EXHIBIT J – PART 1

EXHIBIT J – Materials from H.112 Bill File (2014)

Page

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Genetically Modified Foods

According to the World Health Organization, Genetically Modified Organisms(GMOs) are "organisms in which the genetic material (DNA) has been altered in such a way that does not occur naturally."[1] This technology is also referred to as "genetic engineering", "biotechnology" or "recombinant DNA technology" and consists of randomly inserting genetic fragments of DNA from one organism to another, usually from a different species. For example, an artificial combination of genes that includes a gene to produce the pesticide Cry1Ab protein (commonly known as Bt toxin), originally found in Bacillus thuringiensis, is inserted in to the DNA of corn randomly. Both the location of the transferred gene sequence in the corn DNA and the consequences of the insertion differ with each insertion. The plant cells that have taken up the inserted gene are then grown in a lab using tissue culture and/or nutrient medium that allows them to develop into plants that are used to grow GM food crops.[2]

Natural breeding processes have been safely utilized for the past several thousand years. In contrast, "GE crop technology abrogates natural reproductive processes, selection occurs at the single cell level, the procedure is highly mutagenic and routinely breeches genera barriers, and the technique has only been used commercially for 10 years."[3]

Despite these differences, safety assessment of GM foods has been based on the idea of "substantial equivalence" such that "if a new food is found to be substantially equivalent in composition and nutritional characteristics to an existing food, it can be regarded as safe as the conventional food."[4] However, several animal studies indicate serious health risks associated with GM food consumption including infertility, immune dysregulation, accelerated aging, dysregulation of genes associated with cholesterol synthesis, insulin regulation, cell signaling, and protein formation, and changes in the liver, kidney, spleen and gastrointestinal system.

There is more than a casual association between GM foods and adverse health effects. There is causation as defined by Hill's Criteria in the areas of strength of association, consistency, specificity, biological gradient, and biological plausibility.[5] The strength of association and consistency between GM foods and disease is confirmed in several animal studies.[2,6,7,8,9,10,11]

Specificity of the association of GM foods and specific disease processes is also supported. Multiple animal studies show significant immune dysregulation, including upregulation of cytokines associated with asthma, allergy, and inflammation. [6,11] Animal studies also show altered structure and function of the liver, including altered lipid and carbohydrate metabolism as well as cellular changes that could lead to accelerated aging and possibly lead to the accumulation of reactive oxygen species (ROS). [7,8,10] Changes in the kidney, pancreas and spleen have also been documented. [6,8,10] A recent 2008 study links GM corn with infertility, showing a significant decrease in offspring over time and significantly lower litter weight in mice fed GM corn.[8] This study also found that over 400 genes were found to be expressed differently in the mice fed GM corn. These are genes known to control protein synthesis and modification, cell signaling, cholesterol synthesis, and insulin regulation. Studies also show intestinal damage in animals fed GM foods, including proliferative cell growth9 and disruption of the intestinal immune system.[6]

Regarding biological gradient, one study, done by Kroghsbo, et al., has shown that rats fed transgenic Bt rice trended to a dose related response for Bt specific IgA. [11]

Also, because of the mounting data, it is biologically plausible for Genetically Modified Foods to cause adverse health effects in humans.

In spite of this risk, the biotechnology industry claims that GM foods can feed the world through production of higher crop yields. However, a recent report by the Union of Concerned Scientists reviewed 12 academic studies and indicates otherwise: "The several thousand field trials over the last 20 years for genes aimed at increasing operational or intrinsic yield (of crops) indicate a significant undertaking. Yet none of these field trials have resulted in increased yield in

commercialized major food/feed crops, with the exception of Bt corn."[12] However, it was further stated that this increase is largely due to traditional breeding improvements.

Therefore, because GM foods pose a serious health risk in the areas of toxicology, allergy and immune function, reproductive health, and metabolic, physiologic and genetic health and are without benefit, the AAEM believes that it is imperative to adopt the precautionary principle, which is one of the main regulatory tools of the European Union environmental and health policy and serves as a foundation for several international agreements.[13] The most commonly used definition is from the 1992 Rio Declaration that states: "In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation."[13]

Another often used definition originated from an environmental meeting in the United States in 1998 stating: "When an activity raises threats to the environment or human health, precautionary measures should be taken, even if some cause and effect relationships are not fully established scientifically. In this context, the proponent of an activity, rather than the public, should bear the burden of proof (of the safety of the activity)."[13]

With the precautionary principle in mind, because GM foods have not been properly tested for human consumption, and because there is ample evidence of probable harm, the AAEM asks:

- Physicians to educate their patients, the medical community, and the public to avoid GM foods when possible and provide educational materials concerning GM foods and health risks.
- Physicians to consider the possible role of GM foods in the disease processes of the patients they treat and to document any changes in patient health when changing from GM food to non-GM food.
- Our members, the medical community, and the independent scientific community to gather case studies potentially related to GM food consumption and health effects, begin epidemiological research to investigate the role of GM foods on human health, and conduct safe methods of determining the effect of GM foods on human health.
- For a moratorium on GM food, implementation of immediate long term independent safety testing, and labeling of GM foods, which is necessary for the health and safety of consumers.

(This statement was reviewed and approved by the Executive Committee of the American Academy of Environmental Medicine on May 8, 2009.)

Submitted by Amy Dean, D.O. and Jennifer Armstrong, M.D.

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GM SOY
Sustainable?
Responsible?

A summary of scientific evidence showing that genetically modified (GM) soy and the glyphosate herbicide it is engineered to tolerate are unsustainable from the point of view of farming, the environment, rural communities, animal and human health, and economies

by Michael Antoniou, Paulo Brack, Andrés Carrasco, John Fagan, Mohamed Habib, Paulo Kageyama, Carlo Leifert, Rubens Onofre Nodari, Walter Pengue

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This report was compiled by an international coalition of scientists who hold the view that the complete body of evidence on GM soy and glyphosate herbicide should be made accessible to everyone – government, industry, the media, and the public. The scientists and their contact details are as follows:

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Note: The views expressed in the report, GM Soy: Sustainable? Responsible? are those of the individuals who co-authored the report. There is no implication or claim that they reflect or represent the views of the institutions with which these individuals are or have been affiliated.

The publishers of this report were inspired by the scientists' work on this issue to support its release to the public. The full report and summary of key findings can be downloaded from the publishers' websites:
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EXECUTIVE SUMMARY

Awareness is growing that many modern agricultural practices are unsustainable and that alternative ways of ensuring food security must be found. In recent years, various bodies have entered the sustainability debate by attempting to define the production of genetically modified Roundup Ready® (GM RR) soy as sustainable and responsible.

These include ISAAA, a GM industry-supported group; the research organization, Plant Research International at Wageningen University, the Netherlands, which has issued a paper presenting the arguments for the sustainability of GM RR soy; and the Round Table on Responsible Soy (RTRS), a multi-stakeholder forum with a membership including NGOs such as WWF and Solidaridad and multinational companies such as ADM, Bunge, Cargill, Monsanto, Syngenta, Shell, and BP.

This report assesses the scientific and other documented evidence on GM RR soy and asks whether it can be defined as sustainable and responsible.

GM RR soy is genetically modified to tolerate the herbicide Roundup®, based on the chemical glyphosate. The transgenic modification allows the field to be sprayed with glyphosate, killing all plant life except the crop. GM RR soy was first commercialized in the United States in 1996. Today, GM RR varieties dominate soy production in North America and Argentina and are widely cultivated in Brazil, Paraguay, Uruguay and Bolivia.

Glyphosate is an essential element in the GM RR soy farming system. Because of this, the rapid expansion of GM RR soy production has led to large increases in the use of the herbicide.

The industry claims that glyphosate is safe for people and breaks down rapidly and harmlessly in the environment. But a large and growing body of scientific research challenges these claims, revealing serious health and environmental impacts. The adjuvants (added ingredients) in Roundup increase its toxicity. Harmful effects from glyphosate and Roundup are seen at lower levels than those used in agricultural spraying, corresponding to levels found in the environment.

The widespread spraying of glyphosate on GM RR soy, often carried out from the air, has been linked in reports and scientific research studies to severe health problems in villagers and farmers. A recently published study links glyphosate exposure to birth defects. In some regions around the world, including a GM RR soy-producing region of Argentina, courts have banned or restricted such spraying.

For farmers, GM RR soy has not lived up to industry claims. Studies show that GM RR soy consistently delivers low yields. Glyphosate applications to the crop have been shown in studies to interfere with nutrient uptake, to increase pests and diseases, and to reduce vigour and yield.

The most serious problem for farmers who grow GM RR soy is the explosion of glyphosate-resistant weeds, or "superweeds". Glyphosate-resistant weeds have forced farmers onto a chemical treadmill of using more and increasingly toxic herbicides. In some cases, no amount of herbicide has allowed farmers to gain control of weeds and farmland has had to be abandoned.

The no-till farming model that is promoted as part of the GM RR soy technology package avoids ploughing with the aim of conserving soil. Seed is planted directly into the soil and weeds are controlled with glyphosate applications rather than mechanical methods.

Claims of environmental benefits from the no-till/GM RR soy model have been found to be misleading. The system has added to the glyphosate-resistant weed problem and has been shown to increase the environmental impact of soy production when the herbicides used to control weeds are taken into account. Also, the production of GM RR soy has been found to require more energy than the production of conventional soy.

There are also serious safety questions over the transgenic modifications introduced into GM RR soy. Contrary to claims by the GM industry and its supporters, the US Food and Drug Administration FDA has never approved any GM food as safe. Instead, it de-regulated GM foods in the early 1990s, ruling that they are "substantially equivalent" to non-GM foods and do not need any special safety testing. The ruling was widely recognized as a political decision with no basis in science. In fact, "substantial equivalence" has never been scientifically or legally defined.

Since then, a number of studies have found health hazards and toxic effects associated with GM RR soy. These include cellular changes in organs, more acute signs of ageing in the liver, enzyme function disturbances, and changes in the reproductive organs. While most of these studies were conducted on experimental animals, the findings suggest that GM RR soy may also impact human health. This possibility has not been properly investigated.

Proponents of GM RR soy often justify its rapid expansion on economic grounds. They argue that the crop increases prosperity for farmers, rural communities, and the economy, so it is irresponsible to ask for proper risk assessment.

However, when on-farm economic impacts of growing GM crops are measured, the results are often disappointing. For example, a study for the European Commission found no economic benefit to US farmers from growing GM RR soy over non-GM soy. The most frequently cited benefit for farmers of growing GM RR soy, simplified weed control, is fast unravelling due to the spread of glyphosate-resistant weeds.

Argentina is widely cited as an example of the success

on mice, rats, dogs, monkeys and even humans. Feeding trials were performed over several years to investigate growth, carcinogenicity and effects on reproduction. GM plants have undergone no such investigations.[95]

The genetic engineering process

GM proponents often claim that genetic engineering is simply an extension of conventional plant breeding. But this is untrue. GM uses laboratory techniques to insert artificial gene units into the host plant's genome – a process that would never happen in nature. The artificial gene units are created by joining fragments of DNA from viruses, bacteria, plants and animals. For example, the herbicide-resistant gene in GM RR soy was pieced together from a plant virus, two different soil bacteria, and a petunia plant.

The GM transformation process is imprecise and can cause widespread mutations, resulting in potentially major changes to the plant's DNA blueprint.[96] These mutations can directly or indirectly disrupt the functioning and regulation not just of one or even of several, but of hundreds of genes, leading to unpredictable and potentially harmful effects.[97] These can include the production of unexpected toxic, carcinogenic (cancer-causing), teratogenic (causing birth defects) or allergenic compounds.[98]

Unintended changes in GM crops and foods

Several studies show unintended changes in GM crops as compared with the non-GM parent variety. Changes are seen even when the GM and non-GM equivalent varieties are grown side-by-side in identical conditions and harvested at the same time. This shows that any differences are not caused by environmental conditions but by the GM transformation process.

One such carefully controlled study, comparing GM rice with its non-GM equivalent, showed that the two had different amounts of protein, vitamins, fatty acids, trace elements, and amino acids. The authors concluded that the differences "might be related to the genetic transformation".[99]

Another study comparing Monsanto's GM Bt maize MON810 with non-GM equivalent varieties also found unintended changes resulting from the genetic engineering process. The study found that the GM seeds responded differently to the same environment as compared with their non-GM equivalents, "as a result of the genome rearrangement derived from gene insertion".[100]

In some case, such changes do matter, as health hazards can arise from foreign proteins produced in GM plants as a result of the genetic engineering process.[101] In one study, GM peas fed to mice caused immune responses and the mice became sensitized to other foods, though non-GM peas caused no such reaction. Also, kidney beans naturally containing the gene that was added to the GM peas caused no such reaction. This showed that the mice's reaction to the GM peas was caused by changes brought about by the genetic engineering process.[102]

The GM peas were not commercialized. But unexpected ill effects, including toxic effects and immune responses, have been found in animals fed on GM crops and foods that have been commercialized. These include GM maize[103] [104] [105] [106] and canola/oilseed rape[107] as well as soy.

GM foods and crops: The research climate

When GM RR soy was first approved for commercialization, there were few studies on GM foods and crops. Even today, the body of safety data on GM crops and foods is not as comprehensive as it should be, given that they have been in the food and feed supply for 15 years. This is partly because GM companies use their patent-based control of the crops to restrict research. They often bar access to seeds for testing, or retain the right to withhold permission for a study to be published.[108]

Even pro-GM scientists and media outlets have called for more freedom and transparency in GM crop research. An editorial in Scientific American noted, "Unfortunately, it is impossible to verify that genetically modified crops perform as advertised. That is because agritech companies have given themselves veto power over the work of independent researchers."[109]

There is also a well-documented pattern of GM industry attempts to discredit scientists whose research reveals problems with GM crops.[110] For example, UC Berkeley researchers David Quist and Ignacio Chapela found themselves the targets of an orchestrated campaign to discredit them after they published research showing GM contamination of Mexican maize varieties.[111] An investigation traced the campaign back to the Bivings Group, a public relations firm contracted by Monsanto.[112] [113]

In spite of this restrictive research climate and sometimes in the face of strong industry opposition, hundreds of peer-reviewed studies have been carried out on GM foods and crops. Many assess longer-term impacts such as the widespread rise of glyphosate-resistant weeds around the world. The findings show that GM RR soy is not substantially equivalent to non-GM soy, but differs in its properties, effects on experimental animals, environmental impacts, and in-field performance.

Approval of GM RR soy

Monsanto applied for approval of its GM RR soy for commercialization in 1994. It based its application on research that analyzed the composition, allergenicity, toxicity, and feed conversion of RR soybeans, which, taken together, were intended to demonstrate safety to health.

The research was neither peer-reviewed nor published at the time of the application. Related papers by Monsanto employees appeared only later in scientific journals.[114] [115] [116] [117]

soybean field in eastern South Dakota." Samples were taken from the middle of each field. The GM and non-GM soy supplies for the different diets do not appear to have been tested to confirm that they were in fact different.

Several aspects of the study are poorly described. The authors do not state the amount of non-GM soy that was put into the non-GM diet. They do not specify the amount of either diet consumed by the mice. The feeding protocol, weights of each animal, and growth pattern related to feed intake are not recorded. All these factors are relevant to a rigorous nutritional and toxicological study and yet are not accounted for.

For these reasons, it is not possible to make scientifically defensible claims of safety for GM soy based on this study.

Effects of GM animal feed

Around 38 million tons of soymeal per year are imported into Europe, which mostly goes into animal feed. Around 50–65 percent of this is GM or GM-contaminated, with 14–19 million tons GM-free.

Food products from GM-fed animals do not have to carry a GM label. This is based on assumptions including:

- GM DNA does not survive the animal's digestive process
- GM-fed animals are no different from animals raised on non-GM feed
- meat, fish, eggs and milk from animals raised on GM feed are no different from products from animals raised on non-GM feed.

However, studies show that differences can be found in animals raised on GM RR soy animal feed, compared with animals raised on non-GM feed, and that GM DNA can be detected in the milk and body tissues (meat) of such animals. Findings include:

- DNA from plants is not completely degraded in the gut but is found in organs, blood, and even the offspring of mice.[137] GM DNA is no exception.
- GM DNA from GM maize and GM soy was found in milk from animals raised on these GM crops. The GM DNA was not destroyed by pasteurization.[138]
- GM DNA from soy was found in the blood, organs, and milk of goats. An enzyme, lactic dehydrogenase, was found at significantly raised levels in the heart, muscle, and kidneys of kids fed GM RR soy.[139] This enzyme leaks from damaged cells and can indicate inflammatory or other cellular injury.

Health effects on humans

Very few studies directly examine the effects of GM foods on humans. However, two studies examining possible impacts of GM RR soy on human health found potential problems.

Simulated digestion trials show that GM DNA in GM RR soy can survive passage through the small intestine and would therefore be available for uptake by the intestinal bacteria or cells.[140] Another study showed that GM DNA from RR soy had transferred to intestinal bacteria before the experiment began and continued to be biologically active.[141] These studies were not followed up.

GM proponents often claim that GM DNA in food is broken down and inactivated in the digestive tract. These studies show that this is false.

Nutrient value and allergenic potential

- Studies show that GM RR soy can be less nutritious than non-GM soy and may be more likely to cause allergic reactions:
- GM RR soy had 12–14 per cent lower amounts of isoflavones (compounds that have been found to have anti-cancer effects) than non-GM soy.[142]
- The level of trypsin inhibitor, a known allergen, was 27 per cent higher in raw GM soy varieties.[143]
- GM RR soy was found to contain a protein that differed from the protein in wild type soy, raising the possibility of allergenic properties. One of the human experimental subjects in the study showed an immune response to GM soy but not to non-GM soy.[144]

These findings show that GM soy is not substantially equivalent to non-GM soy.

AGRONOMIC & ENVIRONMENTAL IMPACTS OF GM RR SOY

Many of the promised benefits to farmers of GM crops, including GM RR soy, have not materialized. On the other hand, unexpected problems have arisen.

Yield

The claim that GM crops give higher yields is often uncritically repeated in the media. But this claim is not accurate.

At best, GM crops have performed no better than their non-GM counterparts, with GM RR soy giving consistently lower yields. A review of over 8,200 university-based soybean varietal trials found a yield drag of between 6 and 10 per cent for GM RR soy compared with non-GM soy.[145] Controlled comparative field trials of GM and non-GM soy suggest that half the drop in yield is due to the disruptive effect of the GM transformation process.[146]

Data from Argentina show that GM RR soy yields are the same as, or lower than, non-GM soybean yields.[147] In 2009, Brazilian farmer organization FARSUL published

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

By Michael Antoniou

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 reanalysis 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 (Schrøder 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, IFNy, 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 environmetal 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.

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GMO Myths and Truths

An evidence-based examination of the claims made for the safety and efficacy of genetically modified crops

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earthopensource

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Version 1.3b

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Earth Open Source

Earth Open Source is a not-for-profit organization dedicated to assuring the sustainability, security, and safety of the global food system. It supports agroecological, farmer-based systems that conserve soil, water, and energy and that produce healthy and nutritious food free from unnecessary toxins. It challenges the use of pesticides, artificial fertilizer and genetically modified organisms (GMOs) on the grounds of the scientifically proven hazards that they pose to health and the environment and because of the negative social and economic impacts of these technologies. Earth Open Source holds that our crop seeds and food system are common goods that belong in the hands of farmers and citizens, not of the GMO and chemical industry.

Earth Open Source has established three lines of action, each of which fulfils a specific aspect of its mission:

- Science and policy platform
- Scientific research
- Sustainable rural development.

Science and policy

Because the quality of our food supply is intimately connected with political and regulatory decisions, for example, on pesticides and GMOs, Earth Open Source functions as a science and policy platform to provide input to decision-makers on issues relating to the safety, security and sustainability of our food system.

Earth Open Source has published and co-published several reports that have had impact internationally:

- Roundup and birth defects: Is the public being kept in the dark?
- GM Soy: Sustainable? Responsible?
- Conflicts on the menu: A decade of industry influence at the European Food Safety Authority (EFSA)
- Europe's pesticide and food safety regulators – Who do they work for?

Scientific research and sustainable rural development

Earth Open Source has laboratory and field research projects under way on several continents. Farmer-led agricultural development projects are ongoing in Asia. Details will be released as these projects come to fruition.

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EXECUTIVE SUMMARY

Genetically modified (GM) crops are promoted on the basis of a range of far-reaching claims from the GM crop industry and its supporters. They say that GM crops:

- Are an extension of natural breeding and do not pose different risks from naturally bred crops
- Are safe to eat and can be more nutritious than naturally bred crops
- Are strictly regulated for safety
- Increase crop yields
- Reduce pesticide use
- Benefit farmers and make their lives easier
- Bring economic benefits
- Benefit the environment
- Can help solve problems caused by climate change
- Reduce energy use
- Will help feed the world.

However, a large and growing body of scientific and other authoritative evidence shows that these claims are not true. On the contrary, evidence presented in this report indicates that GM crops:

- Are laboratory-made, using technology that is totally different from natural breeding methods, and pose different risks from non-GM crops
- Can be toxic, allergenic or less nutritious than their natural counterparts
- Are not adequately regulated to ensure safety
- Do not increase yield potential
- Do not reduce pesticide use but increase it
- Create serious problems for farmers, including herbicide-tolerant "superweeds", compromised soil quality, and increased disease susceptibility in crops
- Have mixed economic effects
- Harm soil quality, disrupt ecosystems, and reduce biodiversity
- Do not offer effective solutions to climate change
- Are as energy-hungry as any other chemically-farmed crops
- Cannot solve the problem of world hunger but distract from its real causes – poverty, lack of access to food and, increasingly, lack of access to land to grow it on.

Based on the evidence presented in this report, there is no need to take risks with GM crops when effective, readily available, and sustainable solutions to the problems that GM technology is claimed to address already exist. Conventional plant breeding, in some cases helped by safe modern technologies like gene mapping and marker assisted selection, continues to outperform GM in producing high-yield, drought-tolerant, and pest- and disease-resistant crops that can meet our present and future food needs.

1. THE GENETIC ENGINEERING TECHNIQUE

1.1 **Myth:** Genetic engineering is just an extension of natural breeding
Truth: Genetic engineering is different from natural breeding and poses special risks

GM proponents claim that genetic engineering is just an extension of natural plant breeding. They say that GM crops are no different from naturally bred crops, apart from the inserted foreign GM gene (transgene) and its protein product. But this is misleading. GM is completely different from natural breeding and poses different risks.

Natural breeding can only take place between closely related forms of life (e.g. cats with cats, not cats with dogs; wheat with wheat, not wheat with tomatoes or fish). In this way, the genes that carry information for all parts of the organism are passed down the generations in an orderly way.

In contrast, GM is a laboratory-based technique that is completely different from natural breeding. The main stages of the genetic modification process are as follows:

1. In a process known as tissue culture or cell culture, tissue from the plant that is to be genetically modified is placed in culture.
2. Millions of the tissue cultured plant cells are subjected to the GM gene insertion process. This results in the GM gene(s) being inserted into the DNA of a few of the plant cells in tissue culture. The inserted DNA is intended to re-programme the cells' genetic blueprint, conferring completely new properties on the cell. This process is carried out either by using a device known as a gene gun, which shoots the GM gene into the plant cells, or by linking the GM gene to a special piece of DNA present in the soil bacterium, Agrobacterium tumefaciens. When the A. tumefaciens infects a plant, the GM gene is carried into the cells and can insert into the plant cell's DNA.
3. At this point in the process, the genetic engineers have a tissue culture consisting of hundreds of thousands to millions of plant cells. Some have picked up the GM gene(s), while others have not. The next step is to treat the culture with chemicals to eliminate all except those cells that have successfully incorporated the GM gene into their own DNA.
4. Finally, the few cells that survive the chemical treatment are treated with plant hormones. The

Section at a glance

- Genetic engineering is completely different from natural breeding and entails different risks. The genetic engineering and associated tissue culture processes are imprecise and highly mutagenic, leading to unpredictable changes in the DNA, proteins, and biochemical composition of the resulting GM crop that can lead to unexpected toxic or allergenic effects and nutritional disturbances.
- Foods produced by cisgenic or intragenic methods are as hazardous as any other GM crop.
- It is misleading to compare GM with radiation-induced mutation breeding and to conclude that, as crops bred by the latter method are not tested for safety or regulated, neither should GM crops be tested or regulated. Radiation-induced mutation breeding is potentially even more mutagenic than GM, and at least as destructive to gene expression, and crops produced by this method should be regulated at least as strictly as GM crops.
- It is unnecessary to take risks with GM when conventional breeding – assisted by safe modern gene mapping technologies – is capable of meeting our crop breeding needs.

hormones stimulate these genetically modified plant cells to proliferate and differentiate into small GM plants that can be transferred to soil and grown on.

5. Once the GM plants are growing, the genetic engineer examines them and eliminates any that do not seem to be growing well. He/she then does tests on the remaining plants to identify one or more that express the GM genes at high levels. These are selected as candidates for commercialisation.
6. The resulting population of GM plants all carry and express the GM genes of interest. But they have not been assessed for health and environmental safety or nutritional value. This part of the process will be discussed later in this document.

The fact that the GM transformation process is artificial does not automatically make it undesirable or dangerous. It is the consequences of the procedure that give cause for concern.

Muddying the waters with imprecise terms

GM proponents often use the terminology relating to genetic modification incorrectly to blur the line between genetic modification and conventional breeding.

For example, the claim that conventional plant breeders have been "genetically modifying" crops for centuries by selective breeding and that GM crops are no different is incorrect (see 1.1). The term "genetic modification" is recognised in common usage and in national and international laws to refer to the use of recombinant DNA techniques to transfer genetic material between organisms in a way that would not take place naturally, bringing about alterations in genetic makeup and properties.

The term "genetic modification" is sometimes wrongly used to describe marker-assisted selection (MAS). MAS is a largely uncontroversial branch of biotechnology that can speed up conventional breeding by identifying genes linked to important traits. MAS does not involve the risks and uncertainties of genetic modification and is supported by organic and sustainable agriculture groups worldwide.

Similarly, the term "genetic modification" is sometimes wrongly used to describe tissue culture, a method that is used to select desirable traits or to reproduce whole plants from plant cells in the laboratory. In fact, while genetic modification of plants as carried out today is dependent on the use of tissue culture (see 1.1), tissue culture is not dependent on GM. Tissue culture can be used for many purposes, independent of GM.

Using the term "biotechnology" to mean genetic modification is inaccurate. Biotechnology is an umbrella term that includes a variety of processes in which biological functions are harnessed for various purposes. For instance, fermentation, as used in wine-making and baking, marker assisted selection (MAS), and tissue culture, as well as genetic modification, are all biotechnologies. Agriculture itself is a biotechnology, as are commonly used agricultural methods such as the production of compost and silage.

GM proponents' misleading use of language may be due to unfamiliarity with the field – or may represent deliberate attempts to blur the lines between controversial and uncontroversial technologies in order to win public acceptance of GM.

1.2 **Myth:** Genetic engineering is precise and the results are predictable
Truth: Genetic engineering is crude and imprecise, and the results are unpredictable

GM proponents claim that GM is a precise technique that allows genes coding for the desired trait to be inserted into the host plant with no unexpected effects.

The first step in genetically engineering plants, the process of cutting and splicing genes in the test tube, is precise, but subsequent steps are not. In particular, the process of inserting a genetically modified gene into the DNA of a plant cell is crude, uncontrolled, and imprecise, and causes mutations – heritable changes – in the plant's DNA blueprint.[1] These mutations can alter the functioning of the natural genes of the plant in unpredictable and potentially harmful ways.[2,3] Other procedures associated with producing GM crops, including tissue culture, also produce mutations.[1]

In addition to the unintended effects of mutations, there is another way in which the GM process generates unintended effects. Promoters of GM crops paint a picture of GM technology that is based on a naïve and outdated understanding of how genes work. They propagate the simplistic idea that they can insert a single gene with laser-like precision and insertion of that gene will have a single, predictable effect on the organism and its environment.

But manipulating one or two genes does not just produce one or two desired traits. Instead, just a single change at the level of the DNA can give rise to multiple changes within the organism.[2,4] These changes are known as pleiotropic effects. They occur because genes do not act as isolated units but interact with one another, and the functions and structures that the engineered genes confer on the organism interact with other functional units of the organism.

Because of these diverse interactions, and because even the simplest organism is extremely complex, it is impossible to predict the impacts of even a single GM gene on the organism. It is even more impossible to predict the impact of the GMO on its environment – the complexity of living systems is too great.

In short, unintended, uncontrolled mutations occur during the GM process and complex interactions occur at multiple levels within the organism as a result of the insertion of even a single new gene. For these reasons, a seemingly simple genetic modification can give rise to many unexpected changes in the resulting crop and the foods produced from it. The unintended changes could include alterations in the nutritional content of the food, toxic and allergenic effects, poor crop performance, and generation of characteristics that harm the environment.

These unexpected changes are especially dangerous because they are irreversible. Even the worst chemical pollution diminishes over time as the pollutant is degraded by physical and biological mechanisms. But GMOs are living organisms. Once released into the ecosystem, they do not degrade and cannot be recalled, but multiply in the environment and pass on their GM genes to future generations. Each new generation creates more opportunities to interact with other organisms and the environment, generating even more unintended and unpredictable side-effects.

How can these unintended, unexpected and potentially complex effects of genetic engineering be predicted and controlled? Promoters of GM crops paint a simplistic picture of what is needed for assessing the health and environmental safety of a GMO. But the diversity and complexity of the effects, as well as their unpredictable nature, create a situation where even a detailed safety assessment could miss important harmful effects.

1.3 **Myth:** GM is just another form of mutation breeding and is nothing to worry about
Truth: Mutation breeding brings its own problems and should be strictly regulated

Proponents often describe GM as just another form of mutation breeding, a method of plant breeding which they say has been successfully used for decades and is not controversial. They argue that mutation breeding is regulated no differently than conventional breeding, that genetic modification is just another form of mutation breeding, and that therefore, genetic modification should not be regulated any more stringently than conventional breeding.

However, scientific evidence exposes flaws in this logic.

1.3.1. What is mutation breeding?

The physical form of an organism's genetic blueprint is the sequence of the four "letters" of the genetic alphabet structured within the DNA molecules. Mutations are physical alterations in the sequence of letters within the DNA. Mutation breeding is the process of exposing plant seeds to ionizing radiation (x-rays or gamma rays) or mutagenic chemicals in order to increase the rate of mutation in the DNA.

Just as you can change the meaning of a sentence by changing the sequence of letters in the sentence, you can change the "meaning" of a gene by changing the sequence of letters within the genetic code of the DNA of an organism. A mutagen is a physical or chemical agent that causes such changes.

This process of change in the DNA is known as mutagenesis. Mutagenesis can either completely destroy the function of a gene – that is, "knock out" its function, or it can change the sequence of letters of the genetic code in the gene, causing it to direct the cell to produce one or more proteins with altered function. The resulting plant is called a mutant.

1.3.2. Where did radiation-induced mutation breeding come from?

Mutation breeding using radiation was first seriously investigated in the 1950s, after the US atomic bombing of Japan at the end of World War II in 1945. In the wake of the devastation, there was a desire to find uses for the "peaceful atom" that were helpful to humanity. Atomic Gardens were set up in the US and Europe with the aim of creating high-yielding and disease-resistant crops. They were laid out in a circle with a radiation source in the middle that exposed plants and their seeds to radiation. This would cause mutations in the plants that it was hoped would be beneficial. To the lay population this was euphemistically described as making the plants "atom energized". The results were poorly documented – certainly they do not qualify as scientific research – and it is unclear whether any useful plant varieties emerged from Atomic Garden projects.[5]

Today, radiation-induced mutation breeding is carried out in laboratories, but this branch of plant breeding retains strong links with the nuclear industry. The main database of crop varieties generated using radiation- and chemically-induced mutation breeding is maintained by the UN Food and Agriculture Organisation and the International Atomic Energy Agency.[6] Many studies and reports that recommend radiation-induced mutation breeding are sponsored by organizations that promote nuclear energy.[7][8]

1.3.3. Is mutation breeding widely used?

Mutation breeding is not a widely used or central part of crop breeding, though a few crop varieties have apparently benefited from it. A database maintained by the UN Food and Agriculture Organisation and the International Atomic Energy Agency keeps track of plant varieties that have been generated using mutation breeding and by cross-breeding with a mutant plant.[6] There are only around 3,000 such plant varieties. This number includes not only crop plants but also

ornamental plants.[9] It also includes not only the direct mutant varieties, but also varieties bred by crossing the mutants with other varieties by conventional breeding. Thus the actual number of primary mutant varieties is significantly lower than 3000.

Some commercially important traits have come out of mutation breeding, such as the semi-dwarf trait in rice, the high oleic acid trait in sunflower, the semi-dwarf trait in barley, and the low-linolenic acid trait in canola (oilseed rape).[9,10,11]

But conventional breeding, in contrast, has produced millions of crop varieties. The Svalbard seed vault in the Arctic contains over 400,000 seed varieties,[12] which are estimated to represent less than one-third of our most important crop varieties.[13] So relatively speaking, mutation breeding is of only marginal importance in crop development.

The reason mutation breeding is not more widely used is that the process of mutagenesis is risky, unpredictable, and does not efficiently generate beneficial mutations. Studies on fruit flies suggest that about 70% of mutations will have damaging effects on the functioning of the organism, and the remainder will be either neutral or weakly beneficial.[14]

Because of the primarily harmful effects of mutagenesis, the genetic code is structured to minimize the impacts of mutations and organisms have DNA repair mechanisms to repair mutations. In addition, regulatory agencies around the world are supposed to minimise or eliminate exposure to manmade mutagens.

In plants as well as fruit flies, mutagenesis is a destructive process. As one textbook on plant breeding states, "Invariably, the mutagen kills some cells outright while surviving plants display a wide range of deformities."[15] Experts conclude that most such induced mutations are harmful, and lead to unhealthy and/or infertile plants.[15,16] Occasionally, mutagenesis gives rise to a previously unknown feature that may be beneficial and can be exploited.

The process of screening out undesirable traits and identifying desirable ones for further breeding has been likened to "finding a needle in a haystack".[15] The problem is that only certain types of mutations, such as those affecting shape or colour, are obvious to the eye. These plants can easily be discarded or kept for further breeding as desired. But other more subtle changes may not be obvious, yet may nonetheless have important impacts on the health or performance of the plant. Such changes can only be identified by expensive and painstaking testing.[15]

A report by the UK government's GM Science Review Panel concluded that mutation breeding "involves the production of unpredictable and undirected genetic changes and many thousands, even millions, of undesirable plants are discarded in order to identify plants with suitable qualities for further breeding."[17]

In retrospect, it is fortunate that mutation breeding has not been widely used because that has reduced the likelihood that this risky technology could have generated crop varieties that are toxic, allergenic, or reduced in nutritional value.

1.3.4. How does GM create mutations?

Just as mutation breeding is highly mutagenic, so is the process of creating a GM plant. The GM transformation process involves three kinds of mutagenic effects: insertional mutagenesis, genome-wide mutations, and mutations caused by tissue culture – described below.[1,2]

Insertional mutagenesis

Genetic modification or genetic engineering of an organism always involves the insertion of a foreign gene into the genome (DNA) of the recipient organism. The insertion process is uncontrolled, in that the site of insertion of the foreign gene is random. The insertion of the GM gene (transgene) disrupts the normal sequence of the letters of the genetic code within the DNA of the plant, causing what is called insertional mutagenesis. This can occur in a number of different ways:

- The GM gene can be inserted into the middle of one of the plant's natural genes. Typically this blocks the expression of ("knocks out") the natural gene, destroying its function. Less frequently the insertion event will alter the natural plant gene's structure and the structure

and function of the protein for which it is the blueprint.

- The GM gene can be inserted into a region of the plant's DNA that controls the expression of one or more genes of the host plant, unnaturally reducing or increasing the function of those genes.
- Even if the GM gene is not directly inserted into a host gene or its control region, its mere presence within an active host gene region can alter the ability of that region of the plant's DNA to form chromatin (the combination of DNA and proteins that make up the contents of a cell nucleus) structures that influence the ability of any gene in that region to be expressed. The inserted gene can also compete with host genes for gene expression control elements (comparable to switches that turn the expression of a gene on or off) or regulatory proteins, resulting in marked disturbances in the level and pattern of gene expression.

Since the insertion of the GM gene is an imprecise and uncontrolled process, there is no way of predicting or controlling which of the plant's genes will be influenced – or the extent of the changes caused by the inserted gene.

Genome-wide mutations

In most cases, the insertion process is not clean. In addition to the intended insertion, fragments of the GM gene's DNA can be inserted at other locations in the genome of the host plant. Each of these unintended insertional events may also be mutagenic and can disrupt or destroy the function of other genes in the same ways as the full GM gene.

It is estimated that there is a 53–66% probability that any insertional event will disrupt a gene.[1] Therefore, if the genetic modification process results in one primary insertion and two or three unintended insertions, it is likely that at least two of the plant's genes will be disrupted.

Research evidence also indicates that the GM transformation process can also trigger other kinds of mutations – rearrangements and deletions of the plant's DNA, especially at the site of insertion of the GM gene[1] – which are likely to compromise the functioning of genes important to the plant.

Mutations caused by tissue culture

Three of the central steps in the genetic modification process take place while the host plant cells are being grown in a process called cell culture or tissue culture. These steps include:

(i) The initial insertion of the GM gene(s) into the host plant cells

(ii) The selection of plant cells into which the GM gene(s) have been successfully inserted

(iii) The use of plant hormones to induce cells selected in (ii), above, to develop into plantlets with roots and leaves.

The process of tissue culture is itself highly mutagenic, causing hundreds or even thousands of mutations throughout the host cell DNA.[1,2] Since tissue culture is obligatory to all three steps described above and these steps are central to the genetic engineering process, there is abundant opportunity for tissue culture to induce mutations in the plant cells.

Given the fact that hundreds of genes may be mutated during tissue culture, there is a significant risk that a gene important to some property such as disease- or pest-resistance could be damaged. In another example, a gene that plays a role in controlling chemical reactions in the plant could be damaged, making the crop allergenic or reducing its nutritional value. The effects of many such mutations will not be obvious when the new GM plant is growing in a greenhouse and so genetic engineers will not be able to select them out.

In the process of insertion of a GM gene into the plant host DNA (step i, above), the GM gene is linked with an antibiotic resistance "marker" gene, which will later enable the genetic engineer to identify which plant cells have successfully incorporated the GM gene into their genome.

The host plant cells are then exposed simultaneously to the GM gene and the antibiotic resistance gene in the hope that some will successfully incorporate the GM gene into their genome.

This is a very inefficient process because genomes are designed to exclude foreign genetic material – for example, invading viruses. So out of hundreds of thousands or even millions of host plant cells exposed to the GM gene, only a few will

successfully incorporate the GM gene.

In order to identify and propagate the plant cells that have successfully incorporated the GM gene (step ii, above), biotechnologists usually use antibiotic resistance marker genes. This is because a cell that has successfully integrated the antibiotic resistance marker gene into its genome and expressed that gene is likely also to have integrated the GM gene into its genome and expressed that gene. Therefore, when the population of plant cells is exposed to the antibiotic, the vast majority of recipient plant cells die, leaving only the few cells that have incorporated and expressed the antibiotic resistance marker gene. In almost all cases these cells have also incorporated the GM gene.

Interestingly, this antibiotic-based selection process relies on the expression of the marker gene. This expression is required to make the plant resistant to the antibiotic. If this gene does not express its protein, it will not confer resistance to the antibiotic.

However, not all regions of the plant cell DNA are *permissive* for the gene expression process to take place. In fact, the vast majority of any cell's DNA is *non-permissive*. Because the process of inserting the DNA that contains the GM gene and the antibiotic resistance marker gene is essentially random, most insertions will occur in non-permissive regions of the plant cell DNA and will not result in expression of either the marker gene or the GM gene. Cells in which such insertions have occurred will not survive exposure to the antibiotic. Only when the antibiotic resistance marker gene happens to have been inserted into a permissive region of the plant cell DNA will the cell express the marker gene and be resistant to the antibiotic.

Permissive regions are areas of DNA where genes important to the functioning of the recipient plant cells are present and active. Thus, selection for antibiotic resistance also selects for recipient cells in which the antibiotic marker gene (and by default the GM gene) have inserted into permissive regions of DNA. The consequence of this is an increased likelihood that the insertion of the GM gene and antibiotic marker gene may cause mutational damage to the structure or function of a gene or genes that are important to the function and even the survival of the recipient plant cell.

This means that the GM procedure maximises the likelihood that incorporation of the GM gene will result in insertional mutagenesis to – *damage to* – one or more genes that are active and important to the functioning of the plant host.

We conclude from this analysis of the mechanisms by which the GM process can cause mutations that it is not the elegant and precisely controlled scientific process that proponents claim but depends on a large measure of good fortune as to whether one obtains the desired outcome without significant damage.

1.3.5. Is GM technology becoming more precise?

Technologies have been developed that can target GM gene insertion to a predetermined site within the plant's DNA in an effort to obtain a more predictable outcome and avoid complications that can arise from insertional mutagenesis.[18,19,20,21,22]

However, these GM transformation methods are not fail-safe. Accidental mistakes can still occur. For example, the genetic engineer intends to insert the gene at one particular site, but the gene might instead be inserted at a different site, causing a range of side-effects.

More importantly, plant biotechnologists still know only a fraction of what there is to be known about the genome of any crop species and about the genetic, biochemical, and cellular functioning of our crop species. That means that even if they select an insertion site that they think will be safe, insertion of a gene at that site could cause a host of unintended side-effects that could:

- Make the crop toxic, allergenic or reduced in nutritional value
- Reduce the ability of the GM crop to resist disease, pests, drought, or other stresses
- Reduce the GM crop's productivity or compromise other agronomic traits, or
- Cause the GM crop to be damaging to the environment.

Moreover, because tissue culture must still be carried out for these new targeted insertion methods, the mutagenic effects of the tissue

culture process remain a major source of unintended damaging side-effects.

These newer methods are also cumbersome and time-consuming, so much so that to date no GM crop that is currently being considered by regulators for approval or that is in the commercialisation pipeline has been produced using these targeted engineering methods.

1.3.6. Why worry about mutations caused in genetic engineering?

GM proponents make four basic arguments to counter concerns about the mutagenic aspects of genetic engineering:

"Mutations happen all the time in nature"

GM proponents say, "Mutations happen all the time in nature as a result of various natural exposures, for example, to ultraviolet light, so mutations caused by genetic engineering of plants are not a problem."

In fact, mutations occur infrequently in nature.[9] And comparing natural mutations with those that occur during the GM transformation process is like comparing apples and oranges. Every plant species has encountered natural mutagens, including certain types and levels of ionizing radiation and chemicals, throughout its natural history and has evolved mechanisms for preventing, repairing, and minimising the impacts of mutations caused by such agents. But plants have not evolved mechanisms to repair or compensate for the insertional mutations that occur during genetic modification. Also, the high frequency of mutations caused by tissue culture during the GM process is likely to overwhelm the repair mechanisms of crop plants.

Natural recombination events that move large stretches of DNA around a plant's genome do occur. But these involve DNA sequences that are already part of the plant's own genome, not DNA that is foreign to the species.

"Conventional breeding is more disruptive to gene expression than GM"

GM proponents cite studies by Batista and colleagues[23] and Ahloowalia and colleagues10 to claim that "conventional" breeding is at least as disruptive to gene expression as GM.24 They argue that if we expect GM crops to be tested extensively because of risks resulting from mutations, then governments should require conventionally bred plants to be tested in the same way. But they do not, and experience shows that plants created by conventional breeding are not hazardous. Therefore crops generated by conventional breeding and by genetic engineering present no special risks and do not require special testing.

This argument is based on what appears to be an intentional misrepresentation of the studies of Batista and Ahloowalia. These studies did not compare conventional breeding with GM, but gamma-ray-induced mutation breeding with GM.

The research of Batista and colleagues and Ahloowalia and colleagues actually provides strong evidence consistent with our arguments, above, indicating that mutation breeding is highly disruptive – even more so than genetic modification.

Batista and colleagues found that in rice varieties developed through radiation-induced mutation breeding, gene expression was disrupted even more than in varieties generated through genetic modification. They concluded that for the rice varieties examined, mutation breeding was more disruptive to gene expression than genetic engineering.[23]

Thus, Batista and colleagues compared two highly disruptive methods and concluded that genetic engineering was, in the cases considered in their study, the less disruptive of the two methods.

The GM proponents used the work of Batista and colleagues and Ahloowalia and colleagues to argue that, since mutation breeding is not regulated, genetic modification of crops should not be regulated either. The amusing part of their argument is that they represent the mutation-bred crop varieties as "conventionally bred", not even mentioning that they were generated through exposure to high levels of gamma radiation. They then argue that, since these supposedly "conventionally bred" varieties are disrupted similarly to the GM varieties studied, it was not justified to require GM crop varieties to be subjected to safety assessment when

"conventionally bred" varieties were not.[24]

Their argument only carries weight if the reader is unaware of the biotech proponents' misrepresentation of mutation bred varieties as "conventionally bred". When this fact comes to light, it not only causes their argument to disintegrate, but also exposes what appears to be a willingness to bend the truth to make arguments favouring GM technology. This in turn raises questions regarding the GM proponents' motives and adherence to the standards of proper scientific debate.

Interestingly, the GM proponents' conclusions were diametrically opposite to the conclusions that Batista and colleagues drew from their findings. The researchers concluded that crop varieties produced through mutation breeding and crops produced through genetic engineering should both be subjected to rigorous safety testing.[23]

In contrast, the GM proponents ignored the conclusions of Batista and colleagues and concluded the opposite: that as mutation-bred crops are not currently required to be assessed for safety, GM crops should not be subjected to such a requirement either.

We agree with the conclusions of Batista and colleagues. Although their study does not examine enough GM crop varieties and mutation-bred crop varieties to make generalised comparisons between mutation breeding and genetic engineering, it does provide evidence that both methods significantly disrupt gene regulation and expression, suggesting that crops generated through these two methods should be assessed for safety with similar levels of rigour. The fact that the risks of mutation breeding have been overlooked in the regulations of some countries does not justify overlooking the risks of GM crops.

We recommend that regulations around the world should be revised to treat mutation-bred crops with the same sceptical scrutiny with which GM crops should be treated. In fact, the Canadian government has reached a similar conclusion and requires mutation-bred crops to be assessed according to the same requirements as GMOs produced through recombinant DNA techniques.[25]

"Mutations occurring in genetic modification are no different from those that occur in natural breeding"

GM proponents say that in conventional breeding, traits from one variety of a crop are introduced into another variety by means of a genetic cross. They point out that the result is offspring that receive one set of chromosomes from one parent and another set from the other. They further point out that, during the early stages of development, those chromosomes undergo a process (sister chromatid exchange) in which pieces of chromosomes from one parent are recombined with pieces from the other.

They suggest that the result is a patchwork that contains tens of thousands of deviations from the DNA sequences present in the chromosomes of either parent. They imply that these deviations can be regarded as tens of thousands of mutations, and conclude that because we do not require these crosses to undergo biosafety testing before they are commercialised, we should not require GM crops, which contain only a few genetic mutations, to be tested.

But this a spurious argument, because sister chromatid exchange (SCE) is not the random fragmentation and recombination of the chromosomes of the two parents. Exchanges occur in a precise manner between the corresponding genes and their surrounding regions in the chromosomes donated by the two parents. SCE is not an imprecise, uncontrolled process like genetic modification.

Natural mechanisms at work within the nucleus of the fertilized egg result in precise recombination events between the copy of the maternal copy of gene A and the paternal copy of gene A. Similarly, thousands of other precise recombination events take place between the corresponding maternal and paternal genes to generate the genome that is unique to the new individual.

This is not an example of random mutations but of the precision with which natural mechanisms work on the level of the DNA to generate diversity within a species, yet at the same time preserve, with letter-by-letter exactness, the integrity of the genome.

When a fertilised ovum undergoes sister chromatid exchange as part of conventional breeding, the chromosome rearrangements do not take place in a random and haphazard way, but are precisely guided so that no information is lost. There can be defects in the process, which could lead to mutations. But the process works against defects occurring by employing precise cellular mechanisms that have evolved over hundreds of thousands of years to preserve the order and information content of the genome of the species.

Genetic engineering, on the other hand, is an artificial laboratory procedure that forcibly introduces foreign DNA into the cells of a plant. Once the engineered transgene is in the nucleus of the cells, it breaks randomly into the DNA of the plant and inserts into that site. Furthermore, GM plants do not contain only a few mutations. The GM transformation process produces hundreds or thousands of mutations throughout the plant's DNA.

For these reasons, conventional breeding is far more precise and carries fewer mutation-related risks than genetic engineering.

"We will select out harmful mutations"

GM proponents say that even if harmful mutations occur, that is not a problem. They say that during the genetic engineering process, the GM plants undergo many levels of screening and selection, and the genetic engineers will catch any plants that have harmful mutations and eliminate them during this process.

As explained above, the process of gene insertion during the process of genetic modification selects for engineered GM gene insertion into active gene regions of the host (recipient) plant cell. This means that the process has a high inherent potential to disrupt the function of active genes present in the plant's DNA.

In many cases, the disruption will be fatal – the engineered cell will die and will not grow into a GM plant. In other cases, the plant will compensate for the lost function in some way, or the insertion will occur at a location that seems to cause minimal disruption of the plant cell's functioning. This is what is desired. But just because a plant grows vigorously does not mean that it is safe to eat and safe for the environment. It could have a mutation that causes it to produce substances that harm consumers or to damage the ecosystem.

Genetic engineers do not carry out detailed screening that would catch all potentially harmful plants. They introduce the GM gene(s) into hundreds or thousands of plant cells and grow them out into individual GM plants. If the gene insertion process has damaged the function of one or more plant cell genes that are essential for survival, the cell will not survive this process. So plants carrying such "lethal" mutations will be eliminated. But the genetic engineer is often left with several thousand individual GM plants, each of them different, because:

- The engineered genes have been inserted in different locations within the DNA of each plant
- Other mutations or disturbances in host gene function have occurred at other locations in the plants through the mechanisms described above (1.3.4).

How do genetic engineers sort through the GM plants to identify the one or two that they are going to commercialise? The main thing that they do is to verify that the trait that the engineered transgene is supposed to confer has been expressed in the plant. That is, they do a test that allows them to find the few plants among the many thousands that express the desired trait. Of those, they pick one that looks healthy, strong, and capable of being bred on and propagated.

That is all they do. Such screening cannot detect plants that have undergone mutations that cause them to produce substances that are harmful to consumers or lacking in important nutrients.

It is unrealistic for GM proponents to claim that they can detect all hazards based on differences in the crop's appearance, vigour, or yield. Some mutations will give rise to changes that the breeder will see in the greenhouse or field, but others give rise to changes that are not visible because they occur at a subtle biochemical level or only under certain circumstances. So only a small proportion of potentially harmful mutations will be eliminated by the breeder's superficial

inspection. Their scrutiny cannot ensure that the plant is safe to eat.

Some agronomic and environmental risks will be missed, as well. For instance, during the GM transformation process, a mutation may destroy a gene that makes the plant resistant to a certain pathogen or an environmental stress like extreme heat or drought. But that mutation will be revealed only if the plant is intentionally exposed to that pathogen or stress in a systematic way. Developers of GM crops are not capable of screening for resistance to every potential pathogen or environmental stress. So such mutations can sit like silent time bombs within the GM plant, ready to "explode" at any time when there is an outbreak of the relevant pathogen or an exposure to the relevant environmental stress.

An example of this kind of limitation was an early – but widely planted – variety of Roundup Ready® soy. It turned out that this variety was much more sensitive than non-GM soy varieties to heat stress and more prone to infection.[26]

1.4 **Myth:** Cisgenics/intragenics is a safe form of GM because no foreign genes are involved
Truth: Cisgenic/intragenic foods are just as risky as any other GM food

Some scientists and GM proponents are promoting a branch of genetic engineering they have termed "cisgenics" or "intragenics", which they say only uses genes from the species to be engineered, or a related species. They say that cisgenic/intragenic GMOs are safer and more publicly acceptable than transgenic GMOs, on the claimed grounds that no foreign genes are introduced.[27,28]

An article on the pro-GM Biofortified website, "Cisgenics – transgenics without the transgene", bluntly states the public relations value of cisgenics: "The central theme is to placate the misinformed public opinion by using clever technologies to circumvent traditional unfounded criticisms of biotechnology."[29]

An example of a cisgenic product is the GM "Arctic" non-browning apple, which a Canadian biotechnology company has applied to commercialise in the US and Canada.[30,31]

GM proponents appear to see intragenics/cisgenics as a way of pushing GM foods through regulatory barriers. As two researchers write: "A strong case has been made for cisgenic plants to come under a new regulatory tier with reduced regulatory oversight or to be exempted from GM regulation."[31]

However, in reality, cisgenics and intragenics are just transgenics by another name. The artificial nature of the transgene construct and its way of introduction into the host plant genome make cisgenics/intragenics just as transgenic as cross-species transfers.

The word "intragenic" implies that only genes within the genome of a single species are being manipulated. But although it is possible to isolate a gene from maize, for example, and then put it back into maize, this will not be a purely intragenic process. This is because in order to put the gene back into maize, it is necessary to link it to other sequences at least from bacteria and possibly also from viruses, other organisms, and even synthetic DNA. Inevitably, "intragenic" gene transfer uses sequences from other organisms. Thus, though the gene of interest may be from the same species as the recipient organism, the totality of the genetically modified DNA introduced is not purely intragenic, but is transgenic, in the sense that some of the genetic elements that are introduced into the recipient plant are derived from another species.

The supposedly intragenic Arctic apple is clearly transgenic, in that sequences from foreign species were part of the DNA construct that was introduced into the apple. This introduces major uncertainties into the plant's functioning, because the effects that those foreign sequences might have on the recipient organism are unknown.

The process of inserting any fragment of DNA, whether intragenic or transgenic, into an organism via the GM transformation process carries the same risks. These risks have been discussed in detail, above. Insertion takes place in an uncontrolled manner and results in at least one insertional mutation event within the DNA of the recipient organism. The insertional event will interrupt some sequence within the DNA of the organism and interfere with any natural function that the interrupted DNA may carry. For instance, if the insertion occurs in the middle of a gene, the gene's function could be destroyed. As a result, the organism will lose the cellular function that the gene encodes. In addition, mutagenic effects on the plant's DNA caused by the tissue culture process occur with cisgenics/intragenics, just as with transgenics.

In conclusion, cisgenic/intragenic plants carry the same environmental and health risks as transgenic GM plants.

Conclusion to Section I

GM proponents claim that genetic engineering of crops is no more risky than natural/conventional breeding. But in fact, genetic engineering is different from natural/conventional plant breeding and poses special risks. In particular, the genetic engineering and associated tissue culture processes are highly mutagenic, leading to unpredictable changes in the DNA and proteins of the resulting GM crop that can lead to unexpected toxic or allergenic effects.

Cisgenic or intragenic GM crops pose the same risks as any other transgenic crop. There is nothing "new" about cisgenics/intragenics. These methods only differ from transgenic methods with regard to the choice of organism from which the gene of interest is taken.

Sometimes GM proponents misleadingly compare genetic engineering with radiation-induced mutagenesis, claiming that the latter is natural or conventional breeding, and conclude that genetic engineering is safer than "conventional" breeding. In fact, while radiation-induced mutagenesis is occasionally used in conventional breeding, it is not in itself conventional breeding. Like genetic engineering, radiation-induced mutagenesis is risky and mutagenic. It is not widely used in plant breeding because of its high failure rate. Some researchers have called for crops bred through mutation breeding to be subjected to the same kind of safety assessments as GM crops, a measure required by Canada's food safety authority.

Comparing genetic engineering with radiation-induced mutagenesis and concluding that it is less risky and therefore safe is like comparing a game of Russian Roulette played with one type of gun with a game of Russian Roulette played with another type of gun. Neither game is safe. Both are risky.

A more useful comparison would be between genetic engineering and conventional breeding that does not involve radiation- or chemical-induced mutagenesis. In fact, this is the method that has safely produced the vast majority of our crop plants over the centuries. It is also the method that is most widely used today.

In challenging genetic modification, we are not rejecting science and are not rejecting the most advanced forms of biotechnology, such as marker assisted selection, which speed up and make more precise the methods of conventional breeding. We are only challenging the premature and misguided commercialisation of crops produced using the imprecise, cumbersome, and outdated method of genetic engineering (recombinant DNA technology). Why use these methods when there are better tools in the biotechnology toolbox?

It is unnecessary to take risks with genetic engineering when conventional breeding – assisted by safe modern technologies such as marker assisted selection – is capable of meeting our crop breeding needs (see 7.3.2).

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2. SCIENCE AND REGULATION

2.1 **Myth:** GM foods are strictly regulated for safety **Truth:** GM food regulation in most countries varies from non-existent to weak

"Monsanto should not have to vouchsafe the safety of biotech food. Our interest is in selling as much of it as possible. Assuring its safety is the FDA's job."
– Philip Angell, Monsanto's director of corporate communications[1] (the FDA is the US government's Food and Drug Administration, responsible for food safety)

"Ultimately, it is the food producer who is responsible for assuring safety."
– US Food and Drug Administration (FDA)[2]

"It is not foreseen that EFSA carry out such [safety] studies as the onus is on the [GM industry] applicant to demonstrate the safety of the GM product in question."
– European Food Safety Authority (EFSA)[3]

Industry and some government sources claim that GM foods are strictly regulated.[4] But GM food regulatory systems worldwide vary from voluntary industry self-regulation (in the US) to weak (in Europe). None are adequate to protect consumers' health.

2.1.1. The regulatory process in the USA

"One thing that surprised us is that US regulators rely almost exclusively on information provided by the biotech crop developer, and those data are not published in journals or subjected to peer review... The picture that emerges from our study of US regulation of GM foods is a rubber-stamp 'approval process' designed to increase public confidence in, but not ensure the safety of, genetically engineered foods."
– David Schubert, professor and head, Cellular Neurobiology Laboratory, Salk Institute, commenting on a comprehensive peer-reviewed study of US government's regulation of GMOs that he co-authored[5,6]

GM foods were first commercialised in the US in the early 1990s. The US food regulator, the Food

Section at a glance

- The regulatory regime for GM crops and foods is too weak to protect consumers from the hazards posed by the technology. Regulation is weakest in the US, but is inadequate in most regions of the world, including Europe.
- The US regime assumes that GM crops are safe if certain basic constituents of the GM crop are "substantially equivalent" to those of their non-GM counterparts – a term that has not been legally or scientifically defined. The European regime applies the same concept but terms it "comparative safety assessment". However, when systematic scientific comparisons of a GM crop and its non-GM counterpart are undertaken, the assumption of substantial equivalence is often shown to be false.
- Pro-GM lobbyists have weakened the regulatory process for GM crops, including through the industry-funded group ILSI. No long-term rigorous safety testing of GMOs is required and regulatory assessments are based on data provided by the company that is applying to commercialise the crop.
- The GM industry restricts access to its products by independent researchers, so effects on health and the environment cannot be properly investigated.
- Independent researchers who have published papers containing data that is not supportive of GMOs have been attacked by pro-GM industry groups and individuals (the "shoot the messenger" tactic).

and Drug Adminstration (FDA), allowed the first GM foods onto world markets in spite of its own scientists' warnings that genetic engineering is different from conventional breeding and poses special risks, including the production of new toxins or allergens.[7,8,9,10,11,12] The FDA overruled its scientists in line with a US government decision to "foster" the growth of the GM industry.[13] The FDA formed a policy for GM foods that did not require any safety tests or labelling.

The creation of this policy was overseen by Michael Taylor, FDA's deputy commissioner of policy – a position created especially for Taylor. Taylor was a former attorney for the GM giant Monsanto and later became its vice president for public policy.[14]

Contrary to popular belief, the FDA does not have a mandatory GM food safety assessment process and has never approved a GM food as safe. It does not carry out or commission safety tests on GM foods. Instead, the FDA operates a *voluntary* programme for pre-market review of GM foods. All GM food crops commercialised to date have gone through this review process, but there is no legal requirement for them to do so. Companies that develop GM crops are allowed to put any GMO (genetically modified organism) on the market that they wish, though they can be held liable for any harm to consumers that results from it.

The outcome of the FDA's voluntary assessment is not a conclusion, underwritten by the FDA, that the GMO is safe. Instead, the FDA sends the company a letter to the effect that:

- The FDA acknowledges that the company has provided a summary of research that it has conducted assessing the GM crop's safety
- The FDA states that, based on the results of the research done by the company, the company has concluded that the GMO is safe
- The FDA states that it has no further questions
- The FDA reminds the company that it is responsible for placing only safe foods in the market
- The FDA reminds the company that, if a product is found to be unsafe, the company may be held liable.[15]

Clearly, this process does not guarantee – or even attempt to investigate – the safety of GM foods. While it does not protect the public, it may protect the FDA from legal liability in the event that harm is caused by a GM food.

2.1.2. The sham of substantial equivalence

> *"The concept of substantial equivalence has never been properly defined; the degree of difference between a natural food and its GM alternative before its 'substance' ceases to be acceptably 'equivalent' is not defined anywhere, nor has an exact definition been agreed by legislators. It is exactly this vagueness that makes the concept useful to industry but unacceptable to the consumer...*
>
> *"Substantial equivalence is a pseudo-scientific concept because it is a commercial and political judgment masquerading as if it were scientific. It is, moreover, inherently anti-scientific because it was created primarily to provide an excuse for not requiring biochemical or toxicological tests."*
>
> – Millstone E, Brunner E, Mayer S. Beyond "substantial equivalence". Nature. 1999; 401(6753): 525–526.[16]

The US FDA's approach to assessing the safety of GM crops and foods is based on the concept of substantial equivalence, which was first put forward by the Organisation for Economic Cooperation and Development (OECD), a body dedicated not to protecting public health but to facilitating international trade.[17]

Substantial equivalence assumes that if a GMO contains similar amounts of a few basic components such as protein, fat, and carbohydrate as its non-GM counterpart, then the GMO is substantially equivalent to the non-GMO and no compulsory safety testing is required.

Claims of substantial equivalence for GM foods are widely criticized as unscientific by independent researchers.[18,19,20,21] A useful analogy is that of a BSE-infected cow and a healthy cow. They are substantially equivalent to one another, in that their chemical composition is the same. The only difference is in the shape of a minor component

of a protein (prion), a difference that would not be picked up by a substantial equivalence assessment. Yet few would claim that eating a BSE-infected cow is as safe as eating a healthy cow.

When claims of substantial equivalence have been independently tested, they have been found to be untrue. Using the latest molecular analytical methods, GM crops have been shown to have a different composition to their non-GM counterparts. This is true even when the two crops are grown under the same conditions, at the same time and in the same location – meaning that the changes are not due to different environmental factors but to the genetic modification.

Examples include:

- GM soy had 12–14% lower amounts of cancer-fighting isoflavones than non-GM soy.[22]
- Canola (oilseed rape) engineered to contain vitamin A in its oil had much reduced vitamin E and an altered oil-fat composition, compared with non-GM canola.[23]
- Experimental GM rice varieties had unintended major nutritional disturbances compared with non-GM counterparts, although they were grown side-by-side in the same conditions. The structure and texture of the GM rice grain was affected and its nutritional content and value were dramatically altered. The authors said that their findings "provided alarming information with regard to the nutritional value of transgenic rice" and showed that the GM rice was not substantially equivalent to non-GM.[24]
- Experimental GM insecticidal rice was found to contain higher levels of certain components (notably sucrose, mannitol, and glutamic acid) than the non-GM counterpart. These differences were shown to have resulted from the genetic manipulation rather than environmental factors.[25]
- Commercialised MON810 GM maize had a markedly different profile in the types of proteins it contained compared with the non-GM counterpart when grown under the same conditions.[21]

GM crops also have different effects from their non-GM counterparts when fed to animals (see 3.1.1).

2.1.3. The US government is not impartial regarding GM crops

The US government is not an impartial authority on GM crops. In fact, it has a policy of actively promoting them.[26] Through its embassies and agencies such as the US Department of Agriculture (USDA), the US government pressures national governments around the world to accept GM crops. This has been made clear in a series of diplomatic cables disclosed by Wikileaks, which reveal that:

- The US embassy in Paris recommended that the US government launch a retaliation strategy against the EU that "causes some pain" as punishment for Europe's reluctance to adopt GM crops.[27]
- The US embassy in Spain suggested that the US government and Spain should draw up a joint strategy to help boost the development of GM crops in Europe.[28]
- The US State Department is trying to steer African countries towards acceptance of GM crops.[29,30]

This strategy of exerting diplomatic pressure on national governments to adopt GM crops is undemocratic as it interferes with their ability to represent the wishes of their citizens. It is also inappropriate to use US taxpayers' money to promote products owned by individual corporations.

2.1.4. The regulatory process in Europe and the rest of the world

> *"I suggest to biotechnology companies that they publish results of studies on the safety of GM foods in international peer-reviewed journals. The general population and the scientific community cannot be expected to take it on faith that the results of such studies are favourable. Informed decisions are made on the basis of experimental data, not faith."*
>
> – Domingo JL. Health risks of GM foods: Many opinions but few data. Science. 2000; 288(5472): 1748–1749.[31]

Many governments, including those of the EU, Japan, Australia, and New Zealand, have an

agency that assesses the safety of GM crops. Based on its assessment, the agency recommends approval or rejection of the crop for use in food or animal feed. The final decision is made by the government.

In Europe, the relevant agency is the European Food Safety Authority (EFSA). Typically the EU member states fail to agree on whether to approve a GM crop, with most voting not to approve it, but the vote does not achieve the "qualified majority" required to reject the GMO. The decision passes to the European Commission, which ignores the desires of the simple majority of the member states and approves the GMO.

Worldwide, safety assessments of GMOs by government regulatory agencies are not scientifically rigorous. As in the US, they do not carry out or commission their own tests on the GM crop. Instead, they make decisions regarding the safety of the GMO based on studies commissioned by the very same companies that stand to profit from the crop's approval.

The problem with this system is that industry studies have an inbuilt bias. Published reviews evaluating studies assessing the safety/hazards of various products or technologies have shown that industry-sponsored or industry-affiliated studies are more likely to reach a favourable conclusion about the safety of the product than independent (non-industry-affiliated) studies. The most notorious example is industry studies on tobacco, which succeeded in delaying regulation for decades by sowing confusion about the health effects of smoking and passive smoking.[32] But a similar bias has been found in studies on other products, including pharmaceuticals[33,34] and mobile phones.[35]

Studies on GM crops and foods are no exception. Two published reviews of the scientific literature show that industry-sponsored or –affiliated studies are more likely than independent studies to claim safety for GMOs.[36,37]

Another problem is the frequently unpublished status of the studies that companies submit to regulatory agencies. The fact that they are not published means that they are not readily available for scrutiny by the public or independent scientists.

Unpublished studies fall into the category of so-called "grey literature" – unpublished documents of unknown reliability.

Such grey literature stands in stark contrast with the gold standard of science, peer-reviewed publication. The peer-reviewed publication process, while far from perfect, is the best method that scientists have come up with to ensure reliability. Its strength lies in a multi-step quality control process:

- The editor of the journal sends the study to qualified scientists ("peers") to evaluate. They give feedback, including any suggested revisions, which are passed on to the authors of the study.
- Based on the outcome of the peer review process, the editor publishes the study, rejects it, or offers to publish it with revisions by the authors.
- Once the study is published, it can be scrutinised and repeated (replicated) by other scientists. This repeat-testing is the cornerstone of scientific reliability, because if other scientists were to come up with different findings, this would challenge the findings of the original study.

The lack of availability of industry studies in the past has resulted in the public being deceived over the safety of GMOs. For example, industry's raw data on Monsanto's GM Bt maize variety MON863 (approved in the EU in 2005) were only forced into the open through court action by Greenpeace. Then independent scientists at the France-based research organisation CRIIGEN analysed the raw data and found that Monsanto's own feeding trial on rats revealed serious health effects – including liver and kidney toxicity – that had been hidden from the public.[38,39]

Since this case and perhaps as a result of it, transparency has improved in Europe and the public can obtain industry toxicology data on GMOs from EFSA on request. Only a small amount of information, such as the genetic sequence of the GMO, can be kept commercially confidential.[40]

Similarly, the Australian and New Zealand food safety agency FSANZ makes industry toxicology

data on GMOs available on the Internet. However, in the US, significant portions of the data submitted to regulators are classified as "commercially confidential" and are shielded from public scrutiny.[41]

2.1.5. Europe's comparative safety assessment: Substantial equivalence by another name

Europe's GMO safety assessment process is still evolving. The European Food Safety Authority (EFSA) is in danger of following the US FDA in adopting the concept of substantial equivalence in its GM food assessments – but under another name. EFSA does not use the discredited term "substantial equivalence" but has replaced it with another term with the same meaning: "comparative safety assessment".

The change of name was suggested in a 2003 paper on risk assessment of GM plants.[42] The paper was co-authored by the chair of EFSA's GMO Panel, Harry Kuiper, with Esther Kok. In 2010 Kok joined EFSA as an expert on GMO risk assessment.[43] In their paper, Kuiper and Kok freely admitted that the concept of substantial equivalence remained unchanged and that the purpose of the name change was in part to deflect the "controversy" that had grown up around the term.[42]

At the same time that Kuiper and Kok published their 2003 paper, they were part of a task force of the industry-funded International Life Sciences Institute (ILSI), that was working on re-designing GMO risk assessment.[44] In 2004 Kuiper and Kok co-authored an ILSI paper on the risk assessment of GM foods, which defines comparative safety assessment. The other co-authors include representatives from GM crop companies that sponsor ILSI, including Monsanto, Bayer, Dow, and Syngenta.[45]

EFSA has followed ILSI's suggestion of treating the comparative safety assessment as the basis for GM safety assessments. EFSA has promoted the concept in its guidance documents on assessment of environmental risks of GM plants[46] and of risks posed by food and feed derived from GM animals,[47] as well as in a peer-reviewed paper on the safety assessment of GM plants, food and feed.[48]

In 2012, the EU Commission incorporated the industry- and EFSA-generated concept of the comparative safety assessment into its draft legislation on GM food and feed.[49]

A major problem with the comparative safety assessment is that, as the name suggests, the authorities are beginning to treat it as a safety assessment in itself, rather than as just the first in a series of mandatory steps in the assessment process. In other words, EFSA and the EU Commission are moving towards a scenario in which GM crops and foods that pass this extremely weak initial screening may not be subjected to further rigorous testing.

2.1.6. GM foods would not pass an objective comparative safety assessment

The comparative safety assessment is a weak test of safety. Yet if it were applied objectively, GM crops and foods would not pass even this stage of the risk assessment. This is because as is explained above (2.1.2), many studies on GM crops show that they are not substantially equivalent to the non-GM counterparts from which they are derived. There are often significant differences in the levels of certain nutrients and types of proteins, as well as unexpected toxins or allergens.

GM proponents have sidestepped this problem by widening the range of comparison. Adopting a method originally used by Monsanto in an analysis of its GM soy,[50,51] they no longer restrict the comparator to the GM plant and the genetically similar (isogenic) non-GM line, but recommend as comparators a range of non-isogenic varieties that are grown at different times and in different locations. Some of this "historical" data even dates back to before World War II.[52]

ILSI has created a database of such published data, including data on unusual varieties that have untypically high or low levels of certain components. EFSA experts use this industry database to compare the composition of the GM plant with its non-GM counterparts in GMO risk assessments.[44,53]

If, on the basis of this "comparative safety assessment", EFSA experts judge the GM crop to be equivalent to its non-GM counterpart, it is assumed to be as safe as the non-GM variety.[44,54]

Further rigorous testing is not required, so unexpected changes in the GM crop are unlikely to be identified. Also, testing for interactions between the genome of the GM crop and the environment is not required.

However, the degree of similarity that a GM plant needs to have to non-GM counterparts in order to pass this comparative safety assessment has never been defined. A comparative assessment of a GM plant often reveals significant differences in its composition that are outside the ranges of other non-GM varieties, including historical varieties. But even in these extreme cases, according to scientists who have served on regulatory bodies, the differences are often dismissed as "biologically irrelevant" (see 3.1.2).[52]

Independent scientists have heavily criticised substantial equivalence and comparative safety assessment as the basis of safety assessments of GM crops.[6,16,52,55]

2.1.7. Weakening comparative assessment further by widening the range of comparison

The comparative safety assessment is itself a flawed basis for assessing GMO safety. Yet recent developments have further weakened this already inadequate method.

An EU Directive on the deliberate release of GMOs requires that the comparator against which the GMO should be assessed for safety should be "the non-modified organism from which it is derived".[56] The EU regulation on GM food and feed agrees that the comparator should be the non-GM counterpart.[57]

These rules ensure that the GM crop or food is compared with its genetically similar (isogenic) non-GM counterpart. The comparator will have the same genetic background, but without the GM transformation. So the comparison is correctly designed to find changes caused by the genetic modification process – which should be the purpose of a GMO safety assessment.

Historically, EFSA has followed this principle in its Guidances and Opinions. Yet in a Guidance published in late 2011, EFSA departed from its past practice and EU legislative requirements and broadened the range of acceptable comparators. EFSA even proposed to allow the use of GM plants, rather than the usual non-GM isogenic line, as comparators for stacked events (crops containing multiple GM traits) and concluded that in some cases plants from different species might be accepted as comparators.[58] EFSA's new approach is in line with industry's practices.[50,51] But whether it complies with EU legislation is questionable.

More importantly, the approach of comparing a GM crop with unrelated or distantly related varieties grown at different times and in different locations is scientifically flawed. In order to determine any unintended disruption to gene structure and function and consequent biochemical composition brought about by the GM transformation process, the only valid comparator is the non-GM isogenic line, when the two have been grown side-by-side at the same time. This serves to minimize variables external to the GM transformation process. Thus any changes seen are likely to be caused by the GM process and not some other factor. In contrast, comparisons with unrelated or distantly related varieties grown at different times and in different locations introduce and increase external variables and serve to mask rather than highlight the effects of the GM transformation.

In parallel with the trend of widening the range of comparison in the comparative assessment of a GM plant's composition, industry and regulators have adopted a similar scientifically invalid approach to assessing the health effects of a GMO in animal feeding trials. In these cases, they dismiss statistically significant changes seen in the animals fed the GMO as compared with those fed a non-GM diet as "not biologically meaningful" or "within the range of biological variation" (see 3.1.2–3.1.4 for a detailed discussion of this practice and how it places public health at risk).

These practices run counter to good scientific method and could be described as a way of "disappearing" inconvenient findings of the experiment in question by bringing in data from other experiments until the convenient answer (that the GMO is no different from its non-GM counterpart) is reached.

2.1.8. GM corporations and the US government have designed the GMO regulatory process around the world

The agricultural biotechnology corporations have lobbied long and hard on every continent to ensure that weak assessment models are the norm. Often working through the US government or nonprofit groups, they have provided biosafety workshops and training courses to smaller countries that are attempting to grapple with regulatory issues surrounding GM crops. The result, according to critics, has been models for safety assessment that favour easy approval of GMOs without rigorous assessment of health or environmental risks.

For example, a report by the African Centre for Biosafety (ACB) described how the Syngenta Foundation, a nonprofit organization set up by the agricultural biotechnology corporation Syngenta, worked on "a three-year project for capacity building in biosafety in sub-Saharan Africa". The Syngenta Foundation's partner in this enterprise was the Forum for Agricultural Research in Africa (FARA), a group headed by people with ties to Monsanto and the US government.

The ACB identified the Syngenta Foundation/ FARA project as part of an "Africa-wide harmonisation of biosafety policies and procedures" that will "create an enabling environment for the proliferation of GMOs on the continent, with few biosafety checks and balances".[59]

In India, the US Department of Agriculture led a "capacity building project on biosafety" to train state officials in the "efficient management of field trials of GM crops"[60] – the first step towards full-scale commercialisation. And in 2010, a scandal erupted when a report from India's national science academies recommending release of GM Bt brinjal (eggplant/aubergine) for cultivation was found to contain 60 lines of text copy-pasted almost word for word from a biotechnology advocacy newsletter – which itself contained lines extracted from a GM industry-supported publication.[61]

2.1.9. Independent research on GM foods is suppressed

> *"Unfortunately, it is impossible to verify that genetically modified crops perform as advertised. That is because agritech companies have given themselves veto power over the work of independent researchers... Research on genetically modified seeds is still published, of course. But only studies that the seed companies have approved ever see the light of a peer-reviewed journal. In a number of cases, experiments that had the implicit go-ahead from the seed company were later blocked from publication because the results were not flattering... It would be chilling enough if any other type of company were able to prevent independent researchers from testing its wares and reporting what they find... But when scientists are prevented from examining the raw ingredients in our nation's food supply or from testing the plant material that covers a large portion of the country's agricultural land, the restrictions on free inquiry become dangerous."*
>
> – Editorial, Scientific American[62]

The problem of basing the regulatory process for GM crops on industry studies could be solved by considering independent (non-industry-affiliated) science in the risk assessment. But independent studies on GM foods and crops are rare, because independent research on GM crop risks is not supported financially – and because industry uses its patent-based control of GM crops to restrict independent research. Research that has been suppressed includes assessments of health and environmental safety and agronomic performance of GM crops.[41] Permission to study GM crops is withheld or made so difficult to obtain that research is effectively blocked. For example, researchers are often denied access to commercialised GM seed and the non-GM isogenic lines.

Even if permission to carry out research is given, GM companies typically retain the right to block publication.[63,64] The industry and its allies

also use a range of public relations strategies to discredit and silence scientists who publish research that is critical of GM crops.[65]

In 2009, 26 scientists took the unusual step of making a formal complaint to the US Environmental Protection Agency. They wrote, "No truly independent research can be legally conducted on many critical questions involving these crops."[66] An editorial in Scientific American reported, "Only studies that the seed companies have approved ever see the light of a peer-reviewed journal. In a number of cases, experiments that had the implicit go-ahead from the seed company were later blocked from publication because the results were not flattering."[62]

In response, a new licensing agreement for researchers on GM crops was reached between US Department of Agriculture (USDA) scientists and Monsanto in 2010.[67] However, this agreement is still restrictive, which is not surprising given that the US Department of Agriculture has a policy of supporting GM crops and the companies that produce them (see 2.1.3). Whether this new policy will make a real difference remains to be seen.

The limited amount of independent research that is conducted on GM foods and crops is often ignored or dismissed by regulatory agencies. In addition, findings of harm, whether in independent or industry studies, are explained away as not "biologically relevant" (see 3.1.2).

2.1.10. Researchers who publish studies that find harm from GM crops are attacked

There is a well-documented history of orchestrated attacks by GM proponents on researchers whose findings show problems with GM crops and foods. The GM proponents adopt a variety of tactics, including criticizing the research as "bad science", finding any small flaw or limitation (which almost all studies have) and claiming that this invalidates the findings, and using personal (ad hominem) attacks against the researcher.

Scientific debate is nothing new and is to be welcomed: it is the way that science progresses. A researcher publishes a study; another researcher thinks that certain aspects could have been done better and repeats it with the desired modifications; these findings in turn are added to the database of knowledge for future researchers to build on. But the trend of attempting to silence or discredit research that finds problems with GMOs is unprecedented and has grown in parallel with the commercialization of GM crops.

Unlike in traditional scientific debate, too often the criticism does not consist of conducting and publishing further research that could confirm or refute the study in question. Instead, the critics try to "shout down" the study on the basis of claims that are spurious or not scientifically validated.

There are numerous cases of this pattern, of which the following are just a few examples.

Gilles-Eric Séralini

In 2007 Professor Gilles-Eric Séralini, researcher in molecular biology at the University of Caen and president of the independent research institute CRIIGEN, and his research team published a re-analysis of a Monsanto 90-day rat feeding study that the company had submitted in support of application for the approval of its GM maize MON863. Approval was granted for food and feed in the EU in 2005. Monsanto tried to keep the feeding trial data secret, claiming commercial confidentiality, but it was forced into the open by a court ruling in Germany.

Séralini's re-analysis of the Monsanto data showed that the rats fed GM maize had reduced growth and signs of liver and kidney toxicity. Seralini concluded that it could not be assumed that the maize was safe and asked for such studies performed for regulatory purposes to be extended beyond 90 days so that the consequences of the initial signs of toxicity could be investigated.[38]

After Séralini and his team published this and other papers showing harmful effects from GM crops and the glyphosate herbicide used with GM Roundup Ready crops, he was subjected to a vicious smear campaign. The smears appeared to come from the French Association of Plant Biotechnologies [Association Française des Biotechnologies Végétale] (AFBV), chaired by Marc Fellous.

Séralini believed the researchers Claude Allegre, Axel Kahn, and Marc Fellous were behind

the defamation and intimidation campaign in France. He sued Fellous for libel, arguing that the campaign had damaged his reputation, reducing his opportunities for work and his chances of getting funding for his research.

During the trial, it was revealed that Fellous, who presented himself as a "neutral" scientist without personal interests, and who accused those who criticise GMOs as "ideological" and "militant", owned patents through a company based in Israel. This company sells patents to GM corporations such as Aventis. Séralini's lawyer showed that other AFBV members also have links with agribusiness companies.

The court found in Séralini's favour. The judge sentenced the AFBV to a fine on probation of 1,000 Euros, 1 Euro for compensation (as requested by Séralini) and 4,000 Euros in court fees.[68]

Emma Rosi-Marshall

In 2007 Emma Rosi-Marshall's team published research showing that Bt maize material got into streams in the American Midwest and that when fed to non-target insects, it had harmful effects. In a laboratory feeding study, the researchers fed Bt maize material to the larvae of the caddis fly, an insect that lives near streams. The larvae that fed on the Bt maize debris grew half as fast as those that ate debris from non-GM maize. And caddis flies fed high concentrations of Bt maize pollen died at more than twice the rate of caddis flies fed non-Bt pollen.[69]

Rosi-Marshall was subjected to vociferous criticism from GM proponents, who said that her paper was "bad science". They complained that the study did not follow the type of protocol usual for toxicological studies performed for regulatory purposes, using known doses – even though such protocols are extremely limited and are increasingly coming under fire from independent scientists for being unable to reliably detect risks (see "Jorg Schmidt..." below). Rosi-Marshall replied that her study allowed the caddis flies to eat as much as they wanted, as they would in the wild.[65]

The critics also objected that laboratory findings did not give accurate information about real field conditions. Rosi-Marshall responded that only in the laboratory is it possible to control conditions tightly enough to allow firm conclusions.

Henry I. Miller of the pro-free-market think tank, the Hoover Institution, co-authored and published an opinion piece in which he called the publication of Rosi-Marshall's study an example of the "anti-science bias" of scientific journals and accused the authors of scientific "misconduct". According to Miller, the authors' main crime was failing to mention in their paper another study that concluded that Bt maize pollen did not affect the growth or mortality of filter-feeding caddis flies.[70] Rosi-Marshall responded that she had not cited these findings because they had not been peer-reviewed and published at the time and because they focused on a different type of caddis fly, with different feeding mechanisms from the insects in her study.[65]

Rosi-Marshall and her co-authors stand by their study. In a statement, they said, "The repeated, and apparently orchestrated, ad hominem and unfounded attacks by a group of genetic engineering proponents has done little to advance our understanding of the potential ecological impacts of transgenic corn."[65]

Jorg Schmidt, Angelika Hilbeck and colleagues

A laboratory study (Schmidt, 2009) showed that GM Bt toxins increased the mortality of ladybird larvae that fed on it, even at the lowest concentrations tested. The study showed that claims that Bt toxins are only harmful to a limited number of insect pests and their close relatives are false. Bt toxins were found to harm non-target organisms – ladybirds – that are highly beneficial to farmers.[71] Ladybirds devour pests such as aphids and disease-causing fungi.

Based on this study and over 30 others, in 2009 Germany banned the cultivation of Monsanto's Bt maize MON810, which contains one of the Bt toxins that Schmidt's team found to be harmful.[71] This triggered two opinion pieces that questioned the scientific basis of the German ban[72,73] and one experimental study (Alvarez-Alfageme et al, 2011) that claimed to disprove the adverse effects of the

Bt toxins on ladybird larvae. The authors of the experimental study found no ill effects on ladybird larvae fed on Bt toxins and said that the "apparent harmful effects" found by Schmidt were due to "poor study design and procedures".[74]

The following year a study (Hilbeck et al, 2012) by some of the same authors as Schmidt's study was published, confirming its findings. This study too found that Bt toxins increased the mortality of ladybird larvae. The researchers addressed the main criticisms raised by Alvarez-Alfageme and gave reasons why that study had found no effect. The main reason given was that Alvarez-Alfageme had chosen to expose the ladybird larvae only in a single dose fed over 24 hours and then allowed them to recover by feeding them Bt toxin-free food.[75] Schmidt, on the other hand, had exposed the larvae continuously over 9–10 days[75] – arguably a far more realistic scenario.

In a separate commentary on the controversy, some of the authors of the confirmatory study criticised the confrontational tone, unscientific elements, and "concerted nature" of the three studies that attacked Schmidt's initial findings. The authors noted that the "dogmatic 'refutations'" and "deliberate counter studies" that routinely appear in response to peer-reviewed results on potential harm from GMOs were also a feature of the debate on risks of tobacco, asbestos, the controversial food packaging chemical bisphenol A, and mobile phones.

The authors also criticised the "double standards" that led the European Food Standards Authority (EFSA) to apply excessive scrutiny to papers that draw attention to the risks of GM crops while overlooking obvious deficiencies in studies that assert the safety of GM crops.

For example, Hilbeck and co-authors pointed to major deficiencies in a routine biosafety test performed for regulatory purposes in the approval process of GM Bt crops. The test is supposed to look for toxic effects on non-target insects. In the test protocol, larvae of the green lacewing, a beneficial pest predator insect, are given moth eggs coated in Bt toxin to eat.

However, as Hilbeck and her team noted, lacewing larvae feed by piercing the eggs and sucking out the contents – meaning that they are "truly incapable of ingesting compounds deposited on the exterior of the eggs".

In other words, this supposed biosafety test is incapable of detecting toxic effects even when they occur. This deficiency has even been noted by the US Environmental Protection Agency. And yet, the authors noted, no criticisms of these clearly inappropriate tests were levelled by Alvarez-Alfageme and the other critics of Schmidt's paper.[76]

Arpad Pusztai

On 10 August 1998 the GM debate changed forever with the broadcast of a current affairs documentary on British television about GM food safety. The programme featured a brief but revealing interview with the internationally renowned scientist Dr Arpad Pusztai about his research into GM food safety. Pusztai talked of his findings that GM potatoes had harmed the health of laboratory rats. Rats fed GM potatoes showed excessive growth of the lining of the gut similar to a pre-cancerous condition and toxic reactions in multiple organ systems.

Pusztai had gone public with his findings prior to publication for reasons of the public interest, particularly as the research had been funded by the British taxpayer. He gave his television interview with the full backing of his employers, the Rowett Institute in Scotland.

After the broadcast aired, a political storm broke. Within days, Pusztai had been gagged and fired by the Rowett, his research team was disbanded, and his data was confiscated. His telephone calls and emails were diverted. He was subjected to a campaign of vilification and misrepresentation by pro-GM scientific bodies and individuals in an attempt to discredit him and his research.[77,78,79,80,81]

What caused the Rowett's turnaround? It was later reported that there had been a phone call from Monsanto to the then US president Bill Clinton, from Clinton to the then UK prime minister Tony Blair, and from Blair to the Rowett.[77]

Untruths and misrepresentations about Pusztai's research continue to be circulated by GM proponents. These include claims that no GM potatoes were fed at all and that the experiment

lacked proper controls. Both claims are easily shown to be false by a reading of the study, which subsequently passed peer-review by a larger-than-usual team of reviewers and was published in The Lancet.[82]

Criticisms of the study design are particularly unsound because it was reviewed by the Scottish Office and won a GBP 1.6 million grant over 28 other competing designs. According to Pusztai, it was also reviewed by the BBSRC, the UK's main public science funding body.[77] Even Pusztai's critics have not suggested that he did not follow the study design as it was approved – and if his study had lacked proper controls, the BBSRC and the Scottish Office would have faced serious questions.

Interestingly, one of the critics who claimed that Pusztai's experiment lacked proper controls[83] had previously co-authored and published with Pusztai a study on GM peas with exactly the same design.[84] In fact, the only notable difference between this study and Pusztai's GM potatoes study was the result: the pea study had concluded that the GM peas were as safe as non-GM peas, whereas the potato study had found that the GM potatoes were unsafe.

Pusztai's GM potato research continues to be cited in the peer-reviewed literature as a valid study.

Ignacio Chapela

In 2001 biologist Ignacio Chapela and his colleague David Quist tested native varieties of Mexican maize and found that they had been contaminated by GM genes.[85] The findings were of concern because at the time, Mexico had banned the planting of GM maize out of concern for its native varieties. Mexico is the biological centre of origin for maize and has numerous varieties adapted to different localities and conditions. The GM contamination came from US maize imports.

Chapela started talking to various government officials, who, he felt, needed to know. As his findings were approaching publication in the journal Nature, events took a sinister turn. Chapela said he was put into a taxi and taken to an empty building in Mexico City, where a senior government official threatened him and his family. Chapela had the impression that he was trying to prevent him from publishing his findings.[86,77,87]

Chapela went ahead with publication. Immediately, a virulent smear campaign against him and his research was launched, with most of the attacks appearing on a pro-GM website called AgBioWorld. While AgBioWorld has many scientists among its subscribers, the attacks were not fuelled by scientists, but by two people called Mary Murphy and Andura Smetacek. Murphy and Smetacek accused Chapela of being more of an activist than a scientist. Smetacek suggested that Chapela's study was part of an orchestrated campaign in collusion with "fear-mongering activists (Greenpeace, Friends of the Earth)".[77]

Murphy and Smetacek successfully shifted the focus from the research findings onto the messenger. The journal Science noted the "widely circulating anonymous emails" accusing researchers, Ignacio Chapela and David Quist, of "conflicts of interest and other misdeeds".[88] Some scientists were alarmed at the personal nature of the attacks. "To attack a piece of work by attacking the integrity of the workers is a tactic not usually used by scientists," wrote one.[89]

Investigative research by Jonathan Matthews of the campaign group GMWatch and the journalist Andy Rowell traced Murphy's attacks to an email address owned by Bivings Woodell, part of the Bivings Group, a PR company with offices in Washington, Brussels, Chicago and Tokyo. Bivings developed "internet advocacy" campaigns for corporations and had assisted Monsanto with its internet PR since 1999, when the biotech company identified that the internet had played a significant part in its PR problems in Europe.[77]

Attempts to uncover the identity of Murphy and Smetacek led nowhere, leading the journalist George Monbiot to write an article about the affair entitled, "The fake persuaders: Corporations are inventing people to rubbish their opponents on the internet".[90]

Chapela's finding that GM genes had contaminated native Mexican maize was confirmed by tests carried out by the Mexican government, as reported in Chapela's published study and in a separate article.[85,91]

Conclusion to Section 2

The regulatory regime for GM crops and foods is weakest in the US, the origin of most such crops, but is inadequate in most regions of the world, including Europe. The US regime assumes that GM crops are safe if certain basic constituents of the GM crop are "substantially equivalent" to those of their non-GM counterparts – a term that has not been legally or scientifically defined. The European regime applies the same concept but terms it "comparative safety assessment". But often, when a scientific comparison of a GM crop and its non-GM counterpart is undertaken, the assumption of substantial equivalence is shown to be false, as unexpected differences are found.

No regulatory regime anywhere in the world requires long-term or rigorous safety testing of GM crops and foods. Regulatory assessments are based on data provided by the company that is applying to commercialise the crop – the same company that will profit from a positive assessment of its safety.

The regulatory procedure for GM crops is not independent or objective. The GM crop industry, notably through the industry-funded group, the International Life Sciences Institute (ILSI), has heavily influenced the way in which its products are assessed for safety. ILSI has successfully promoted ideas such as the comparative safety assessment, which maximize the chances of a GMO avoiding rigorous safety testing and greatly reduce industry's costs for GMO authorisations.

The GM crop industry restricts access to its products by independent researchers, so their effects on human and animal health and the environment cannot be properly investigated. Independent researchers who have published papers containing data that is not supportive of GMOs have been attacked by the industry and pro-GMO groups and individuals. This has had a chilling effect on the debate about GM crops and has compromised scientific progress in understanding their effects.

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3. HEALTH HAZARDS OF GM FOODS

3.1 **Myth:** GM foods are safe to eat
Truth: Studies show that GM foods can be toxic or allergenic

> *"Most studies with GM foods indicate that they may cause hepatic, pancreatic, renal, and reproductive effects and may alter haematological [blood], biochemical, and immunologic parameters, the significance of which remains to be solved with chronic toxicity studies."*
>
> – Dona A, Arvanitoyannis IS. Health risks of genetically modified foods. Crit Rev Food Sci Nutr. 2009; 49: 164–175[1]

There are three possible sources of adverse health effects from GM foods:

- The GM gene product – for example, the Bt toxin in GM insecticidal crops – may be toxic or allergenic
- The GM transformation process may produce mutagenic effects, gene regulatory effects, or effects at other levels of biological structure and function that result in new toxins or allergens and/or disturbed nutritional value
- Changes in farming practices linked to the use of a GMO may result in toxic residues – for example, higher levels of crop contamination with the herbicide Roundup are an inevitable result of using GM Roundup Ready® crops (see Sections 4, 5).

Evidence presented below and in Sections 4 and 5 suggests that problems are arising from all three sources – throwing into question GM proponents' claims that GM foods are as safe as their non-GM counterparts.

3.1.1. Feeding studies on laboratory and farm animals

Feeding studies on laboratory and farm animals show that GM foods can be toxic or allergenic:

Section at a glance

- Peer-reviewed studies have found harmful effects on the health of laboratory and livestock animals fed GMOs. Effects include toxic and allergenic effects and altered nutritional value.
- Most animal feeding studies on GMOs have only been short-term or medium-term in length. What is needed are long-term and multi-generational studies on GMOs to see if the worrying changes commonly reported in short- and medium-term studies develop into serious disease. Such studies are not required by government regulators.
- Industry and regulators dismiss findings of harm in animal feeding trials on GMOs by claiming they are "not biologically significant" or "not biologically relevant" – scientifically meaningless terms that have not been properly defined.
- No GM nutritionally enhanced (biofortified) foods are available on the market. In contrast, conventional plant breeding has successfully and safely produced many biofortified foods.
- The most-hyped GM nutritionally enhanced food, Golden Rice, aimed at combating vitamin A deficiency, has wasted millions in development funds – yet has not been proven safe to eat and is still not ready for the market. Meanwhile, proven and inexpensive solutions to vitamin A deficiency are available and only need proper funding to be more widely applied.

- Rats fed GM tomatoes developed stomach lesions (sores or ulcers).[2,3] This tomato, Calgene's Flavr Savr, was the first commercialized GM food.
- Mice fed GM peas (not subsequently commercialized) engineered with an insecticidal

protein (alpha-amylase inhibitor) from beans showed a strong, sustained immune reaction against the GM protein. Mice developed antibodies against the GM protein and an allergic-type inflammation response (delayed hypersensitivity reaction). Also, the mice fed on GM peas developed an immune reaction to chicken egg white protein. The mice did not show immune or allergic-type inflammation reactions to either non-GM beans naturally containing the insecticide protein, to egg white protein fed with the natural protein from the beans, or to egg white protein fed on its own. The findings showed that the GM insecticidal protein acted as a sensitizer, making the mice susceptible to developing immune reactions and allergies to normally non-allergenic foods. This is called immunological cross-priming. The fact that beans naturally containing the insecticidal protein did not cause the effects seen with the peas that expressed the transgenic insecticidal protein indicated that the immune responses of the mice to the GM peas were caused by changes in the peas brought about by the genetic engineering process. In other words, the insecticidal protein was changed by the GM process so that it behaved differently in the GM peas compared with its natural form in the non-GM beans – and the altered protein from the GM peas stimulated a potent immune response in the mice.[4]

- Mice fed GM soy showed disturbed liver, pancreas and testes function. The researchers found abnormally formed cell nuclei and nucleoli in liver cells, which indicates increased metabolism and potentially altered patterns of gene expression.[5,6,7]
- 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.[8]
- Rabbits fed GM soy showed enzyme function disturbances in kidney and heart.[9]
- Female rats fed GM soy showed changes in uterus and ovaries compared with controls fed organic non-GM soy or a non-soy diet. Certain ill effects were found with organic soy as well as GM soy, showing the need for further investigation into the effects of soy-based diets (GM and non-GM) on reproductive health.[10]
- A review of 19 studies (including industry's own studies submitted to regulators in support of applications to commercialise GM crops) on mammals fed with commercialised GM soy and maize that are already in our food and feed chain found consistent toxic effects on the liver and kidneys. Such effects may be markers of the onset of chronic disease, but long-term studies, in contrast to these reported short- and medium-term studies, would be required to assess this more thoroughly. Unfortunately, such long-term feeding trials on GMOs are not required by regulators anywhere in the world.[11]
- Rats fed insecticide-producing MON863 Bt maize 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 maize is safe and that long-term studies were needed to investigate the consequences of these effects.[12]
- Rats fed GM Bt maize over three generations suffered damage to liver and kidneys and alterations in blood biochemistry.[13]
- A re-analysis of Monsanto's own rat feeding trial data, submitted to obtain approval in Europe for three commercialised GM Bt maize varieties, MON863, MON810, and NK603, concluded that the maize varieties 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.[14] The data suggest that approval of these GM maize varieties should be withdrawn because they are not substantially equivalent to non-GM maize and are toxic.
- Old and young mice fed GM Bt maize showed a marked disturbance in immune system cells and in biochemical activity.[15]
- Rats fed GM MON810 Bt maize showed clear signs of toxicity, affecting the immune system, liver and kidneys.[14,15]
- Female sheep fed Bt GM maize over three generations showed disturbances in the functioning of the digestive system, while

their lambs showed cellular changes in the liver and pancreas.[16]

- GM Bt maize DNA was found to survive processing and was detected in the digestive tract of sheep. This raises the possibility that the antibiotic resistance gene in the maize could move into gut bacteria, an example of horizontal gene transfer.[17] In this case, horizontal gene transfer could produce antibiotic-resistant disease-causing bacteria ("superbugs") in the gut.
- Rats fed GM oilseed rape developed enlarged livers, often a sign of toxicity.[18]
- Rats fed GM potatoes showed excessive growth of the lining of the gut similar to a pre-cancerous condition and toxic reactions in multiple organ systems.[19,20]
- Mice fed a diet of GM Bt potatoes or non-GM potatoes spiked with natural Bt toxin protein isolated from bacteria showed abnormalities in the cells and structures of the small intestine, compared with a control group of mice fed non-GM potatoes. The abnormalities were more marked in the Bt toxin-fed group. This study shows not only that the GM Bt potatoes caused mild damage to the intestines but also that Bt toxin protein is not harmlessly broken down in digestion, as GM proponents claim, but survives in a functionally active form in the small intestine and can cause damage to that organ.[21]
- Rats fed GM rice for 90 days had a higher water intake as compared with the control group fed the non-GM isogenic line of rice. The GM-fed rats showed differences in blood biochemistry, immune response, and gut bacteria. Organ weights of female rats fed GM rice were different from those fed non-GM rice. The authors claimed that none of the differences were "adverse", but they did not define what they mean by "adverse". Even if they had defined it, the only way to know if such changes are adverse is to extend the length of the study, which was not done. The authors conceded that the study "did not enable us to conclude on the safety of the GM food".[22]
- 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.[23]
- A study on rats fed GM Bt rice found a Bt-specific immune response in the non-GM-fed control group as well as the GM-fed groups. The researchers concluded that the immune response in the control animals was due to their inhaling particles of the powdered Bt toxin-containing feed consumed by the GM-fed group. The researchers recommended that for future tests involving Bt crops, GM-fed and control groups should be kept separate.[24] This indicates that animals can be extremely sensitive to very small amounts of GM proteins, so even low levels of contamination of conventional crops with GMOs could be harmful to health.

In these studies, a GM food was fed to one group of animals and its non-GM counterpart was fed to a control group. The studies found that the GM foods were more toxic or allergenic than their non-GM counterparts.

3.1.2. Masking statistical significance through the concept of "biological relevance"

Study findings such as those described above have made it increasingly difficult for GM proponents to continue to claim that there are no differences between the effects of GM foods and their non-GM counterparts – clearly, there are.

To sidestep this problem, the GM industry and its allies have shifted their argument to claim that statistically significant effects, such as those found in the above studies, are not "biologically relevant".

The concept of biological relevance was initially promoted by the industry-funded group, the International Life Sciences Institute (ILSI), and affiliates to argue against regulatory restrictions

on toxic chemicals.[25] But increasingly, it has been extended to the field of GM crops and foods.[26] Biological relevance offers a route through which GM proponents can admit that feeding experimental animals a GM diet can cause statistically significant observable effects, but at the same time argue that these effects are not important.

However, this argument is scientifically indefensible. Biological relevance with respect to changes brought about by GM foods has never been properly defined, either scientifically or legally. Most feeding trials on GM foods, including those carried out by industry to support applications for GM crop commercialisation, are not long-term but medium-term studies of only 30–90 days long and therefore cannot thoroughly assess the safety of GMOs.

In order to determine whether changes seen in these medium-term studies are biologically relevant, the researchers would have to:

- Define in advance what "biological relevance" means with respect to effects found from feeding GM crops
- Extend the current study design from a medium-term to a long-term period. In the case of rodent studies, this would be two years – the approximate duration of their life-span[11]
- Examine the animals closely to see how the changes found in 90-day studies progress – for example, if they disappear or develop into disease or premature death
- Analyze the biological relevance of the changes in light of the researchers' definition of the term
- Carry out additional reproductive and multigenerational studies to determine effects on fertility and future generations.

Since these steps are not followed in cases where statistically significant effects are dismissed as not "biologically relevant", assurances of GM food safety founded on this line of argument are baseless.

In parallel with "biological relevance", a trend has grown of claiming that statistically significant effects of GM feed on experimental animals are not "adverse".[27] However, the term "adverse" is not defined and the experiments are not extended to check whether changes are the first signs of disease. So again, the term is technically meaningless.

We conclude that GM proponents and regulatory bodies should cease masking findings of statistically significant effects from GM crops through poorly defined and scientifically indefensible concepts.

3.1.3. How misuse of "biological relevance" places public health at risk: Monsanto GM maize study

In 2007 a team led by Professor Gilles-Eric Séralini at the independent research institute CRIIGEN in France published a new analysis of a rat feeding study conducted by Monsanto with one of its GM maize varieties.

The maize, called MON863, was approved for feed and feed in Europe in 2005–2006.[28] The maize was approved partly on the basis of the Monsanto study, which, however, could not be scrutinized by independent scientists and the public because the raw data were kept hidden on claimed grounds of commercial confidentiality. Only after court action in Germany forced disclosure of Monsanto's data could Séralini and associates conduct their analysis.[12]

Séralini's team found that according to Monsanto's own data, rats fed GM maize over a 90-day period had signs of liver and kidney toxicity. Also, the GM-fed rats had statistically significant differences in weight from those fed non-GM maize control diets. The GM-fed females had higher concentrations of certain fats in their blood, and excretion of certain minerals was disturbed in GM-fed males.[12]

However, all statistically significant effects found in Monsanto's study were dismissed by the European Food Safety Authority (EFSA) in its favourable safety assessment of the maize. They claimed that the statistically significant effects were not "biologically meaningful".[29,30] EFSA and GM proponents cited differences in response to the GM feed between male and female animals, claiming that toxic effects should be the same in both sex groups.[11,31,32,33] However, this is scientifically indefensible as toxins with hormone-disrupting properties are

well known to have different effects on males and females.[34,35]

Séralini commented on the dangerous trend of dismissing statistically significant effects by claiming lack of biological relevance in a 2011 review of the scientific literature assessing the safety of GM crops: "The data indicating no biological significance of statistical effects in comparison to controls have been published mostly by [GM crop development] companies from 2004 onwards, and at least 10 years after these GMOs were first commercialized round the world". Séralini called the trend a matter of "grave concern".[11]

After years of heavy criticism of the "biological relevance" tactic by independent scientists and a member of the European Parliament,[36,11,37] in late 2011 EFSA issued an Opinion on the relationship between statistical significance and biological relevance.[38]

But EFSA's Opinion failed to give a rigorous scientific or legal definition of what makes a statistically significant finding not "biologically relevant". Instead, it allowed industry to come to its own conclusion on whether changes found in an experiment are "important", "meaningful", or "may have consequences for human health". These are vague concepts for which no measurable or objectively verifiable endpoints are defined. Thus they are a matter of opinion, not science.

Moreover, the lack of a sound definition of biological relevance means that regulators have no strong scientific or legal grounds to disagree with industry's claim that a statistically significant finding is not biologically relevant. This, in effect, makes GMOs impossible to regulate.

The conclusions of the EFSA Opinion are not surprising, given that it is authored by several affiliates of the industry-funded group, the International Life Sciences Institute (ILSI), including Harry Kuiper[39] (also the chair of EFSA's GMO panel), Josef Schlatter, and Susan Barlow.[40] Because ILSI is funded by GM crop development companies, allowing ILSI affiliates to write EFSA's scientific advice on how to assess the safety of GM foods and crops is akin to allowing a student to write his or her own examination paper – or allowing scientists to review their own papers submitted for publication!

3.1.4 Masking statistical significance through the concept of "normal variation"

Studies often find statistically significant differences in the composition of GM foods compared with their isogenic or near-isogenic non-GM counterparts (isogenic means genetically identical except for the one gene of interest, in this case the genetically modified gene). Studies also find statistically significant differences in animals fed a GM crop variety compared with animals fed the isogenic or near-isogenic variety.

However, GM proponents consistently dismiss these statistically significant differences in the experiment under examination by claiming that they are within the "normal variation range" or "within the range of biological variation".

This tactic was used in a review of animal feeding studies on GMOs (the review included many of the studies summarised in this report). In spite of the significant differences found in the GM-fed animals, the reviewers used the concept of normal variation to argue that "GM plants are nutritionally equivalent to their non-GM counterparts and can be safely used in food and feed".[26]

However, this is scientifically unjustifiable. GM proponents define the "normal range of variation" by collecting values from many different studies carried out across a wide range of dates, using different experimental conditions and measurement methods. The result is a set of numbers that vary widely, but there is no scientific justification for including those numbers in the same dataset. On the contrary, there is much justification for excluding most of the values.

By using a dataset with such an unjustifiably wide range of variation, GM proponents are able to hide the genuine and meaningful differences between the GMO of interest and the valid controls – namely the isogenic or near-isogenic variety.

This is an attempt to minimize statistically significant differences brought about by the

GM process by artificially widening the range of values compared beyond what can be scientifically justified. The practice runs counter to the aim of scientific experiments, which are designed to minimise variables. According to rigorous scientific practice, in any single experiment, the scientist manipulates just one variable in order to test its effect. In this way, any changes that are observed can be traced to a probable single cause.

In an animal feeding trial with GMOs, the manipulated variable is the GMO. One group of animals, the "treated" group, is fed a diet containing the GMO. Another group, the control group, is fed a similar diet, with the only difference being that it has not been subject to genetic modification. All conditions of the experiment outside the GM component of the treated group's diet must be the same. Within this tightly controlled setup, any changes seen in the treated group are likely to be caused by the GM process.

Therefore, in any experiment to discover the effects of a GMO in an animal feeding trial, the only valid comparator is the control group within that same experiment (the concurrent control).

By comparing the treated group with a wide variety of control groups from other experiments (sometimes called "historical control data"), GM proponents are masking the effects of the GM process or GM diet, as any GM-related changes will disappear in the "noise" of the changes caused by many variables.

3.1.5. Regulators currently do not require long-term tests on GMOs

In order to detect health effects caused over time in humans eating GM foods, long-term (chronic) animal feeding trials are needed. But currently, no long-term tests on GM crops or foods are required by regulatory authorities anywhere in the world. Reproductive and multigenerational tests, which are necessary to discover effects of GM crops or foods on fertility and future generations, are also not required.[11]

This contrasts with the testing requirements for pesticides or drugs, which are far more stringent. Before a pesticide or drug can be approved for use, it must undergo one-year, two-year, and reproductive tests on mammals.[12] Yet GM foods escape such testing, in spite of the fact that virtually all commercialised GM foods are engineered either to contain an insecticide or to tolerate being sprayed with large amounts of herbicide, so they are likely to contain significant amounts of pesticides.

The longest tests that are routinely conducted on GM foods for regulatory assessments are 90-day rodent feeding trials, and even these are not compulsory.[11] While a 2012 EU draft regulation requests such tests for the time being, the wording is weak and foresees a situation in which they are not required.[41] Also, the type of findings that would trigger a regulatory requirement for such tests has not been specified.[42]

Such 90-day rodent trials are medium-term (subchronic) tests that correspond to only a few years in terms of human lifespan and are too short to show long-term effects such as organ damage or cancer.[43] In addition, too few animals are used in these industry tests to reliably detect harmful effects.

In spite of these serious shortcomings of regulatory tests, statistically significant harmful effects have been found even in industry's own 90-day rodent feeding trials. The most common effects observed are signs of toxicity in the liver and kidney, which are the major detoxifying organs and the first to show evidence of chronic disease.[11]

These observations are consistently interpreted by GM proponents and regulators as "not biologically significant" or as "within the range of normal variation", using the spurious arguments described in Section 3.1.4, above.

3.1.6. Stacked-trait crops are less rigorously tested than single-trait crops

Most GM crops currently on the market and in the approvals pipeline are not single-trait crops but stacked-trait crops. "Stacked-trait" means that several GM traits are combined in one seed. For example, GM SmartStax maize has eight GM traits: six for insect resistance (Bt) and two for tolerance to different herbicides.

Biotech companies have had to resort to

developing multi-trait crops because of the failure of single traits. For example (see Section 5):

- Bt crops have fallen victim to secondary insect pests
- Pests have developed resistance to single Bt toxins
- Weeds have become increasingly resistant to glyphosate, the herbicide that most first-generation GM crops were engineered to tolerate.

Stacked GM crops present more of a regulatory challenge than single-trait crops because of the risk of unexpected interactions between the different GM genes introduced into the crop – and between the introduced GM genes and the genes of the host plant. There is also the risk of combination effects from toxins produced in the plant and/or pesticide residues. In short, the addition of multiple traits to a single crop increases the risk of unexpected and unintended harmful side-effects.

However, stacked-trait GM crops are even less rigorously investigated for possible health effects than single-trait GM crops. While the US does not require toxicological testing of any GM crops, Europe currently requires 90-day toxicological testing on single-trait GM crops. But in the case of stacked-trait crops, the EU food safety authority EFSA does not require toxicity testing of the final stacked-trait crop, believing that it can assess the toxicity of the final stacked-trait crop by looking at industry test findings on the single-event crops that were used to develop it.[44]

This move is irresponsible in the extreme, as such an assessment process depends on a series of assumptions, not on scientific testing. It fails to look at the actual effects of the mixed transgenes and their products within the crop.

3.2 **Myth:** EU research shows GM foods are safe **Truth:** EU research shows evidence of harm from GM foods

GM proponents often refer to research studies that they claim show the safety of GM foods. However, on closer examination, these same studies raise serious safety concerns. A related tactic is to claim that regulatory authorities have pronounced GM foods to be safe – when the regulators' actual statements are either equivocal or are based on industry-provided data.

The success of these tactics relies on the likelihood that few people will look at the source documents that are claimed to provide evidence for the safety of GM foods.

An example of such misrepresented sources is a group of fifty research projects funded by the European Union around the topic of the safety of GMOs for animal and human health and the environment. The results of the projects were published in 2010 by the European Commission in a report called *A Decade of EU-Funded GMO Research (2001–2010)*.[45]

This EU report has been seized upon by GM proponents and some EU officials to bolster their claims that GMOs are safe. Some says that EU regulators have also reached this conclusion, based on the projects' findings. Those who have cited the projects in this way include:

- The GM industry lobby group ISAAA[46]
- Jonathan Jones, a British Monsanto-connected scientist[47][48]
- Nina Fedoroff, former science and technology adviser to US secretary of state Hillary Clinton[49]
- Máire Geoghegan-Quinn, European Commissioner for research, innovation and science.[50]

Oddly, however, ISAAA, Jones, and Federoff do not cite any actual studies performed by the EU researchers. They do not even cite the findings or conclusions of the Commission's report on the studies, *A Decade of EU-Funded GMO Research*.

Instead, they cite a quote from an EU Commission press release announcing the publication of its report. The press release cites Máire Geoghegan-Quinn, European Commissioner for research, innovation and science, as stating that the EU research projects provided "no scientific evidence associating GMOs with higher risks for the environment or

for food and feed safety than conventional plants and organisms".[50]

But it was not the studies' findings, nor even the Commission's report of those findings, but Geoghegan-Quinn's soundbite about the report that found its way into the GM proponents' statements. Closer examination of the case shows why.

Tracing the evidence back to its source, we examine first the report to which Geoghegan-Quinn was referring in her quote: *A Decade of EU-Funded GMO Research*. Of the fifty research projects discussed in the report, just ten are listed as relating to safety aspects of GM foods.[45]

However, within those ten projects, there is astonishingly little data of the type that could be used as credible evidence regarding the safety or harmfulness of GM foods. Such evidence would normally consist of long-term animal feeding studies comparing one group of animals fed a diet containing one or more GM ingredients with a control group fed a diet containing the same ingredients in non-GM form. Instead, the studies examine such topics as risk assessment of GM foods, methods of testing for the presence and quantity of GMOs in food and feed, and consumer attitudes to GM foods.

This data is not relevant to assessing the safety of any GM food. In fact, the report makes clear that the food safety research studies were not designed to do so – though taxpayers would be entitled to ask why the Commission spent 200 million Euros of public money[45] on a research project that failed to address this most pressing of questions about GM foods. Instead, the research studies were designed to develop "safety assessment approaches for GM foods".[45] One of the published studies carried out under the project confirms that the aim was "to develop scientific methodologies for assessing the safety" of GM crops.[23]

Nonetheless, a few animal feeding studies with GM foods were carried out as part of the EU project. It is difficult to work out how many studies were completed, what the findings were, and how many studies passed peer review and were published, because the authors of the EU Commission report fail to reference specific studies to back up their claims. Instead, they randomly list references to a few published studies in each chapter of the report and leave the reader to guess which statements refer to which studies.

In some cases it is unclear whether there is any published data to back up the report's claims. For example, a 90-day feeding study on hamsters is said to show that "the GM potato was as safe as the non-GM potato", but no reference is given to any published study or other source of data, so there is no way of verifying the claim.[45]

Our own search of the literature uncovered three published studies on GM food safety that were carried out as part of SAFOTEST, one of the ten food safety-related projects. Our examination of these studies below reveals that, contrary to the claims of GM proponents and Commissioner Geoghegan-Quinn, they do not show the safety of GM food but rather give cause for concern.

3.2.1. Poulsen (2007)[22]

A feeding trial on rats fed GM rice found significant differences in the GM-fed group as compared with the control group fed the non-GM parent line of rice. These included a markedly higher water intake by the GM-fed group, as well as differences in blood biochemistry, immune response, and gut bacteria. Organ weights of female rats fed GM rice were different from those fed non-GM rice. Commenting on the differences, the authors said, "None of them were considered to be adverse". But they added that this 90-day study "did not enable us to conclude on the safety of the GM food."[22]

In reality, a 90-day study is too short to show whether any changes found are "adverse" (giving rise to identifiable illness). Yet no regulatory body requires GM foods to be tested for longer than this subchronic (medium-term) period of 90 days.

The study found that the composition of the GM rice was different from that of the non-GM parent, in spite of the fact that the two rice lines were grown side-by-side in identical conditions. This is clear evidence that the GM transformation process had disrupted gene structure and/or function in the GM variety, making it non-substantially equivalent to the non-GM line.

3.2.2. Schrøder (2007)[23]

A study on rats fed GM Bt rice found significant differences in the GM-fed group of rats as compared with the group fed the non-GM isogenic line of rice. These included differences in the distribution of gut bacterial species – the GM-fed group had 23% higher levels of coliform bacteria. There were also differences in organ weights between the two groups, namely in the adrenals, testis and uterus. The authors concluded that the "possible toxicological findings" in their study "most likely will derive from unintended changes introduced in the GM rice and not from toxicity of Bt toxin" in its natural, non-GM form.[23]

The study found that the composition of the GM rice was different from that of the non-GM isogenic (with the same genetic background but without the genetic modification) variety in levels of certain minerals, amino acids, and total fat and protein content.[23] These differences were dismissed on the basis that they were within the range reported for all varieties of rice in the literature. However, comparing the GM rice to genetically distinct, unrelated rice varieties is scientifically flawed and irrelevant. It serves only to mask the effects of the GM process (see 2.1.5, 2.1.6, 2.1.7).

Despite this flawed approach, the level of one amino acid, histidine, was markedly higher in the GM rice compared with the non-GM isogenic variety and outside the variability range for any rice.[23] Does this matter? No one knows, as the required investigations have not been carried out. What is known is that in other studies on rats, an excess of histidine caused rapid zinc excretion[51] and severe zinc deficiency.[52]

In addition, the level of the fatty acid, stearic acid, was below the value reported in the literature for any rice.[23]

3.2.3. Kroghsbo (2008)[24]

A study on rats fed GM Bt rice found a Bt-specific immune response in the non-GM-fed control group as well as the GM-fed groups. This unexpected finding led the researchers to conclude that the immune response in the control animals must have been due to their inhaling particles of the powdered Bt toxin-containing feed consumed by the GM-fed group. The researchers recommended that for future tests on Bt crops, GM-fed and control groups should be kept in separate rooms or with separate air handling systems.[24]

3.2.4. Conclusion on the SAFOTEST studies

The three SAFOTEST studies examined above provide no evidence of safety for GM foods and crops. On the other hand, they provide evidence that:

- Over a decade after GM foods were released into the food and feed supplies, regulators still have not agreed on methods of assessing them for safety
- The GM foods tested were markedly different in composition from their non-GM counterparts – probably due to the mutagenic or epigenetic (producing changes in gene function) effects of the GM process
- The GM foods tested caused unexpected, potentially adverse effects in GM-fed animals that should be investigated further in long-term tests
- The authors were not able to conclude that the GM foods tested were safe.

3.3 **Myth:** Those who claim that GM foods are unsafe are being selective with the data, since many other studies show safety **Truth:** Studies that claim safety for GM crops are more likely to be industry-linked and therefore biased

> *"In a study involving 94 articles selected through objective criteria, it was found that the existence of either financial or professional conflict of interest was associated [with] study outcomes that cast genetically modified products in a favourable light."*
> – Diels J, et al. Association of financial or professional conflict of interest to research outcomes on health risks or nutritional assessment studies of genetically modified products. Food Policy. 2011; 36: 197–203

When it comes to hazardous products, the bias of industry-sponsored or industry-linked studies is well documented. Every time industry-linked studies are compared with studies on the same product from the independent (non-industry-linked) scientific literature, the same verdict is reached: industry studies are biased towards conclusions of safety for the product.

The best known example is tobacco industry studies, which successfully delayed regulation for decades by manufacturing doubt and controversy about the negative health effects of smoking and passive smoking.[53] More recently, studies sponsored by the pharmaceutical and mobile phone industry have been shown to be more likely to portray their products in a favourable light than non-industry-funded studies.[54,55,56]

The case of GM crops is no different. Reviews of the scientific literature on the health risks of GM foods demonstrate that the studies that show safety are more likely to be industry-linked and are therefore inherently biased:

- A review of 94 published studies on health risks and nutritional value of GM crops found that they were much more likely to reach favourable[53] conclusions when the authors were affiliated with the GM industry than when the authors had no industry affiliation. In the studies where there was such a conflict of interest, 100% (41 out of 41) reached a favourable conclusion on GMO safety.[57]
- A literature review of GM food safety studies found that most studies concluding that GM foods are as nutritious and safe as non-GM counterparts were performed by the developer companies or associates.[58]

In spite of the fact that industry-linked studies have been shown to be biased, approvals for GM crops are based solely on such industry studies.

Another tactic used by GM proponents is to point to lists of studies which they say show that GM foods are safe, but which actually show nothing of the sort. An example is on the GMO Pundit blog site, which claims that the over 400 cited studies "document the general safety and nutritional wholesomeness of GM foods and feeds."[59]

But closer examination reveals:

- Most of the studies cited are not safety studies on GM foods. In other words, they are not animal feeding studies that look for health effects in animals fed GM foods. Some are compositional studies that compare the levels of certain major nutrients, such as fat or protein, in a GM crop with levels in a non-GM crop. Others are feed conversion studies that measure how efficiently a livestock animal converts GM feed into a food product, such as meat or milk.
- Many of the studies, on examination of the actual data, show problems with GM foods. These include unintended differences in a GM food compared with the non-GM counterpart and harmful effects in animal feeding trials. In fact, some of these studies are cited in this report as evidence that GM foods are not safe. Readers are encouraged to examine the original studies, where available, and form their own conclusions.

In contrast with these lists on GM proponents' websites, the two peer-reviewed literature reviews cited above identified and evaluated the studies that specifically examine the food safety and nutritional value of GM foods. Their conclusions were clear: industry-linked studies are more likely to conclude safety, whereas independent studies are more likely to find problems.[57,58]

3.4 **Myth:** GM foods have been proven safe for human consumption **Truth:** The few studies that have been conducted on humans show problems

GM foods are not properly tested for human safety before they are released for sale.[60,19] The only published studies that have directly tested the safety of GM foods for human consumption found potential problems but were not followed up:

- In a study on human volunteers fed a single GM soybean meal, GM DNA survived processing and was detected in the digestive tract. There was evidence of horizontal gene transfer to gut bacteria.[61,62] Horizontal gene transfer is a process by which DNA is transferred from one organism to another through mechanisms other than reproductive mechanisms. These mechanisms enable one organism to incorporate into its own genome genes from another organism without being the offspring of that organism.
- In a study on humans, one of the experimental subjects showed an immune response to GM soy but not to non-GM soy. GM soy was found to contain a protein that was different from the protein in non-GM soy. This shows that GM foods could cause new allergies.[63]
- A GM soy variety modified with a gene from Brazil nuts was found to react with antibodies present in blood serum taken from people known to be allergic to Brazil nuts. Based on current immunological knowledge, this observation indicates that this soy variety would produce an allergic reaction in people allergic to Brazil nuts.[64]
- A study conducted in Canada detected significant levels of the insecticidal protein, Cry1Ab, which is present in GM Bt crops, circulating in the blood of pregnant women and in the blood supply of their foetuses, as well as in the blood of non-pregnant women.[65] How the Bt toxin protein got into the blood (whether through food or another exposure route) is unclear and the detection method used has been disputed by defenders of GM crops. Nevertheless, this study raises questions as to why GM Bt crops are being commercialised widely, when existing research raises serious concerns about their safety and yet no systematic effort is under way to replicate and thereby assess the validity of that research.

These studies should be followed up with controlled long-term studies and GM foods and crops should not be commercialised in the absence of such testing.

3.5 **Myth:** No one has ever been made ill by a GM food **Truth:** There is no scientific evidence to support this claim

GM proponents claim that people have been eating GM foods in the United States for 16 years without ill effects. But this is an anecdotal, scientifically untenable assertion, as no epidemiological studies to look at GM food effects on the general population have ever been conducted.

Furthermore, there are signs that all is not well with the US food supply. Reports show that food-related illnesses increased two- to ten-fold in the years between 1994 (just before GM food was commercialized) and 1999.[66,67] No one knows if there is a link with GM foods because they are not labelled in the US and consumers are not monitored for health effects.

Under the conditions existing in the US, any health effects from a GM food would have to meet very specific and unusual conditions before they would be noticed. They would have to:

- Occur soon after eating a food that was known to be GM – in spite of its not being labelled – so that the consumer could establish a causal correlation between consumption and the harmful effect. Increases in diseases like cancer, which has a long latency period, would not be traceable to a GM food.
- Cause symptoms that are different from common diseases. If GM foods caused a rise in common diseases like allergies or cancer, nobody would know what caused the rise.
- Be dramatic and obvious to the naked eye or to the consumer of the GMO. No one examines a person's body tissues with a microscope for harm after they eat a GM food. But just this type of examination is needed to give early warning of problems such as pre-cancerous changes.

In addition, health effects would have to be recorded and reported by a centralized body that the public knew about and that could collate data as it came in and identify correlations. Currently, there is no such monitoring body in place anywhere.

Moderate or slow-onset health effects of GM foods could take decades to become apparent through epidemiological studies, just as it took decades for the damaging effects of trans fats (another type of artificial food) to be recognised. Slow-poison effects from trans fats have caused millions of premature deaths across the world.[68] To detect important but subtle effects on health, or effects that take time to appear (chronic effects), long-term controlled studies on large populations would be needed.

3.5.1. Two outbreaks of illness linked to GM foods

Two high-profile cases have emerged in which a GM food was suspected of causing illness in people. In both cases, industry and regulators denied that genetic engineering was the cause, but an examination of the evidence gives no such reassurance.

L-tryptophan

In 1989 in the US, a food supplement, L-tryptophan, produced using GM bacteria, was found to be toxic, killing 37 people and permanently disabling over 1500 others.[69,70,71] The resulting disease was named eosinophilia myalgia syndrome (EMS). Symptoms included an overproduction of white blood cells called eosinophils, severe myalgia (muscle pain), and in some cases, paralysis.

The L-tryptophan that affected people was traced back to a single source, a Japanese company called Showa Denko. In July 1990, a study published in the Journal of the American Medical Association mentioned that Showa Denko had introduced a new genetically engineered bacterium, called Strain V, in December 1988, a few months before the main epidemic hit.[71]

There is an ongoing debate about whether the toxin's presence in the L-tryptophan was due to genetic engineering or to Showa Denko's sloppy manufacturing processes. The company had made changes to its carbon filtration purification process before the toxic contaminant was discovered.

However, the authors of a 1990 study on the outbreak published in the New England Journal of Medicine (NEJM) pointed out that blaming a failure in the carbon filtration process leaves unanswered the question of how the toxin got into the product in the first place.[72] This was a novel toxin that was not found in other companies' L-tryptophan products. The authors of the study, which was sponsored by the US Centers for Disease Control, noted that the new GM bacterial strain introduced by the manufacturer before the outbreak "may have produced larger quantities" of the toxin than earlier strains.[72]

One of the study's co-authors, Dr Michael Osterholm, an epidemiologist at the Minnesota Department of Health, commented in a press article of August 1990 that the new bacterial strain "was cranked up to make more L-tryptophan and something went wrong. This obviously leads to that whole debate about genetic engineering."[73]

Following Osterholm's comment, a number of press articles appeared voicing doubts about the safety of genetic engineering. The FDA took on the role of exonerating genetic engineering from blame for the EMS epidemic. An article in Science magazine quoted FDA official Sam Page as saying that Osterholm was "propagating hysteria". Tellingly, Page added, "The whole question: Is there any relation to genetic engineering? is premature – *especially given the impact on the industry*"[74] (our emphasis).

Osterholm countered: "Anyone who looks at the data comes to the same conclusion [that there may be a link with genetic engineering]... I think FDA doesn't want it to be so because of the implications for the agency."[74]

James Maryanski, FDA biotech policy coordinator, blamed the EMS epidemic on Showa Denko's changes to the purification process.[75] Maryanski also said that genetic engineering could not have been solely or even chiefly responsible for EMS because cases of the illness had been reported for several years before Showa Denko introduced its genetically engineered bacterial Strain V in December 1988.[76]

However, a study published in 1994 shows that this argument is misleading. Showa Denko had named its bacterial strain "V" because there had been four previous strains of the bacterium. Over a period of years, Showa Denko had progressively introduced more genetic modifications into the bacteria used in its manufacturing process. It began using Strain V in December 1988, shortly before the EMS main outbreak in 1989.[69] But it had begun using its first genetically modified strain, Strain II, in 1984, according to lawyers who took on the cases of EMS sufferers.[77] This timescale means that Showa Denko's genetically engineered bacteria could have been responsible for the EMS epidemic.

The FDA responded to the crisis by claiming that all L-tryptophan was dangerous and temporarily banning all L-tryptophan from sale.[78] But a study sponsored by the Centers for Disease Control said if that were true, then "all tryptophan products of equal dose produced from different companies should have had the same [effect]". The study concluded that this was not the case, since out of six manufacturers of L-tryptophan, only Showa Denko's product was clearly associated with illness.[79]

If Showa Denko's L-tryptophan were produced today, it would have to be assessed for safety, since it was derived from GM bacteria. However, since this L-tryptophan was greater than 99% pure and devoid of DNA, it would be passed as substantially equivalent to the same substance obtained from non-GM organisms. In other words, the tests that would be required to detect novel toxins of this type would be seen as unnecessary and no labelling would be required. So the same tragedy would result.[80]

StarLink maize

In 2000 in the US, people reported allergic reactions, some of them severe, to maize (corn) products. A GM Bt maize called StarLink was found to have contaminated the food supply. Regulators had allowed StarLink to be grown for animal feed and industrial use but had not approved it for human food because of suspicions that the Bt insecticidal protein it contained, known as Cry9C, might cause allergic reactions.

The number of people who reported allergic reactions to maize products is not known because

there was no centralized reporting system. The US Food and Drug Administration (FDA) analyzed reports that had reached it and asked the US Centers for Disease Control (CDC) to investigate just 28 cases that met its criteria. CDC carried out tests on blood serum taken from these people but concluded that the findings did not provide evidence that the allergic reactions were associated with the Cry9C protein.[81]

However, there were problems with the CDC investigation, many of which were identified by the researchers themselves. For example, the control group of serum was obtained from blood samples taken before the 1996 release of StarLink. Yet this serum showed a more dramatic allergic response to Cry9C than the serum from people who had reported allergic reactions to StarLink.[81] The researchers stated that this is common in samples that have been frozen and stored, as the control samples had been. But they expressed no concern that this would skew the results towards a false conclusion of no effect from StarLink. Neither did they replace the problem control samples with more reliable ones – for example, samples freshly taken from people who were unlikely to have been exposed to StarLink.

CDC's test and findings were reviewed by a panel convened by the US Environmental Protection Agency (EPA) – which criticised them on several grounds. The panel pointed out that the CDC researchers had isolated the Cry9C protein from E. coli bacteria rather than from StarLink maize. So the protein tested would have been different from the Cry9C protein suspected of causing allergic reactions.[82] Specifically, the Cry9C protein from E. coli bacteria would have lacked sugar molecules, which would have been attached through a process called glycosylation to the same protein derived from maize. Glycosylation can be crucial in eliciting an allergic reaction. CDC's use of the incorrect protein invalidates its analysis and conclusions.

The seriousness of CDC's error in using E. coli- rather than maize-derived Cry9C protein is graphically illustrated by the study on GM peas containing an insecticidal protein from beans (see 3.1.1).[4] The study found marked changes in the pattern of sugar molecules on the insecticidal protein expressed in the GM peas, as compared with its native form in beans. The authors concluded that this change in the nature and structure of the sugar molecules was the reason why the GM insecticidal protein caused immune and allergic-type inflammation reactions in mice.

This case shows that it is necessary to derive the GM protein being studied from the GM crop rather than an unrelated source, as sugar molecule patterns will differ and the potential to cause immune and allergic reactions could vary significantly between the two.

Furthermore, the EPA panel criticised the CDC's test for its lack of proper controls. It also questioned the methodology and sensitivity of the test used. The EPA panel concluded, "The test, as conducted, does not eliminate StarLink Cry9C protein as a potential cause of allergic symptoms". The panel's verdict was that there is a "medium likelihood" that the Cry9C protein is an allergen.[82]

3.5.2. Conclusion

Claims that no one has been made ill by a GM crop or food are scientifically unjustifiable, since no epidemiological studies have been carried out. However, the cases of L-trypophan produced with GM bacteria and StarLink maize give cause for concern.

3.6 **Myth:** GM Bt insecticidal crops only harm insects and are harmless to animals and people
Truth: GM Bt insecticidal crops pose hazards to people and animals that eat them

Many GM crops are engineered to produce Bt toxin, a type of insecticide. Bt toxin in its natural, non-GM form is derived from a common soil bacterium and is used as an insecticidal spray in chemically-based and organic farming.

Regulators have approved GM Bt crops on the assumption that the GM Bt toxin is the same as the natural Bt toxin, which they say has a history of safe use. They conclude that GM crops engineered to contain Bt insecticidal protein must also be harmless.

But this is false, for the following reasons:

- Natural Bt toxin is not necessarily the same as the Bt toxin expressed by GM Bt plants. The Bt toxin protein in GM plants may be truncated or otherwise modified. For example, there is at least a 40% difference between the toxin in Bt176 maize (formerly commercialised in the EU, now withdrawn) and natural Bt toxin.[11] Such changes can mean that they have very different effects on people or animals that eat them. Prions (the folded proteins found in BSE-infected cows), venoms, and hormones, are all proteins, but are far from harmless.[83]
- The natural Bt toxin used in insecticidal sprays behaves differently in the environment from the Bt toxin produced in GM plants. Natural Bt breaks down rapidly in daylight and only becomes active (and toxic) in the gut of the insect that eats it. It does not persist in the environment and so is unlikely to find its way into animals or people that eat the crop. With GM Bt crops, however, the plant is engineered to express the Bt toxin protein in active form in every cell. In other words, the plant itself becomes a pesticide, and people and animals that eat the plant are eating a pesticide.
- Even natural Bt toxin has been found to have negative health effects. In farm workers, exposure to Bt sprays was found to lead to allergic skin sensitisation and immune responses.[84] And laboratory studies found that natural Bt toxin has ill effects on mammals, producing a potent immune response and enhancing the immune response to other substances.[85,86,87]
- Safety tests for regulatory purposes are generally not carried out on the Bt toxin protein as expressed in the GM plant. The Bt toxin protein that is tested is usually derived from genetically engineered E. coli bacteria, as GM companies find it too difficult and expensive to extract enough Bt toxin from the GM crop itself. As we have seen, the GM process gives rise to unexpected changes in the desired protein, so it cannot be assumed that the Bt toxin protein derived from E. coli bacteria is the same as the protein derived from the GM plant that people and animals will eat. Indeed, the US Environmental Protection Agency, in its review of the commercialised Monsanto GM maize MON810, said it produces a "truncated" version of the protein – in other words, a protein that is not the same as the natural form.[60] Such changes can make a protein more toxic or allergenic.

3.6.1. Bt toxin does not only affect insect pests

GM proponents claim that the Bt toxin engineered into GM Bt crops only affects the target pests and is harmless to mammals, including people or animals that eat the crops.[88] Based on this assumption, regulators do not require human toxicity studies on GM Bt crops.

But the assumption is incorrect. In a 2012 test-tube (in vitro) study, genetically engineered Bt toxins were found to be toxic to human cells. One type of Bt toxin killed human cells at the dose of 100 parts per million. The findings showed that GM Bt toxin does affect humans, contrary to claims from the GM lobby and regulators.[83]

The GM lobby responded by saying that in vitro studies do not accurately reflect what happens in a living human or animal that eats GM Bt crops. But

other independent studies have found that GM Bt crops have adverse effects when fed to laboratory animals. Findings include:

- Toxic effects on the small intestine, liver, kidney, spleen, and pancreas[12,14,16,21,40]
- Disturbances in the functioning of the digestive system[16]
- Reduced weight gain[12]
- Immune system disturbances.[15]

Aside from laboratory animals and human cells, GM Bt crops have been found to have toxic effects on butterflies and other non-target insects,[89,90,91] beneficial pest predators,[92,93] bees,[94] and aquatic[95,96] and soil organisms[97] (see section 4).

It is premature to say that the toxic effects associated with GM Bt crops are due to the Bt toxin from the crops. The effects may be due to one or more of the following causes:

- The Bt toxin as produced in the GM crop
- New toxins produced in the Bt crop by the GM process, and/or
- Residues of herbicides or chemical insecticides used on the Bt crop. Many Bt crops have added herbicide-tolerant traits,[98] making it likely that herbicide residues will be found on them.

3.6.2. Bt toxin protein may not be broken down harmlessly in the digestive tract

GM proponents claim that the Bt toxin insecticidal protein in GM plants is broken down in the digestive tract and so cannot get into the blood or body tissues to cause toxic effects.

But digestion is generally an incomplete process and studies show that Bt toxin protein is not always fully broken down:

- A study on cows found that Bt toxins from GM maize MON810 were not completely broken down in the digestive tract.[99]
- A study simulating human digestion found that the Bt toxin protein was highly resistant to being broken down in realistic stomach acidity conditions and still produced an immune response.[100]
- 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.[65] Questions have been raised about the validity of the detection method, but further investigation is needed before Bt crops can be claimed to be safe for humans.

3.6.3. Conclusion

Studies on GM Bt crops show that Bt toxin is not specific to a narrow range of insect pests but can affect a wide variety of non-target organisms. Taken together, the studies on GM Bt crops and natural Bt toxin raise the possibility that eating GM crops containing Bt toxin may cause toxic or allergic reactions and/or sensitise people to other food substances.

3.7 **Myth:** GM foods are properly tested for ability to cause allergic reactions
Truth: No thorough allergenicity testing is conducted on GM foods

> *"There is more than a casual association between GM foods and adverse health effects.... Multiple animal studies show significant immune dysregulation, including upregulation of cytokines [protein molecules involved in immune responses] associated with asthma, allergy, and inflammation."*
>
> – American Academy of Environmental Medicine[101]

Most food allergies are caused by a reaction to a protein in a food. The DNA of an organism contains instructions for making proteins. Genetic engineering changes the DNA of a food, and that altered DNA can in turn can create new proteins. Therefore, GM foods could create new allergies in two ways: the new proteins could cause allergic reactions (be "allergens") themselves, or the new proteins could sensitise people to existing food proteins.

The website GMO Compass, which is run by the public relations firm Genius GmbH, claims that GM plants pose no greater risk than new varieties of crops obtained through conventional breeding, or the importation of new exotic foods, which can also result in new allergens appearing in the diet.[102]

But independent scientists disagree. A 2003 review states that compared with conventional breeding, GM has a "greater potential to introduce novel proteins into the food supply" and increase the likelihood of allergic reactions.[103] This was confirmed by a rare study on humans, in which one of the experimental subjects showed an immune response to GM soy but not to non-GM soy. GM soy was found to contain a protein that was different from the protein in the non-GM variety.[63]

3.7.1. The EU system for assessing GM plants for allergenicity

Under European law, GM plants must be assessed for their potential to cause allergies before they are allowed onto the market. Proponents claim that any potentially allergenic GM foods are likely to be caught by these regulatory checks. The GMO Compass website calls these assessments "rigorous" and adds, "If a GM plant is found to contain a potential allergen, its chances of receiving approval in the EU are slim to none."[102,104]

But in reality, the European regulatory process, though stronger than the US process, has no rigorous system for assessing the allergenic potential of GM foods. This is largely because reliable scientific tests to predict allergenicity have not been developed.

The process that EU regulators use to assess the allergenicity of GM foods[102,105] is based on a system proposed in 2001 by the Food and Agriculture Organisation of the United Nations and the World Health Organisation.[106] This system was actually designed by two GM industry-funded groups, the International Life Sciences Institute (ILSI), and the International Food Biotechnology Council (IFBC), as the FAO/WHO freely states.[106]

The process begins with a comparison of the protein that the GM plant is designed to produce with known allergenic proteins. Depending on the outcome of this initial assessment, further investigations can include:

- Tests to see if the new protein reacts with the blood serum of sensitive individuals
- Artificial stomach tests to see if the protein is broken down easily (if it is, it is thought unlikely to be an allergen)
- Animal feeding trials.[102]

3.7.2. Why the allergy assessment process is ineffective

Independent scientists have stated that the EU's allergenicity assessment is unlikely to reliably predict whether a GM food is likely to cause allergic reactions.

The most important reason is that the new protein that is assessed in the regulatory process is normally not the protein as expressed in the whole GM plant. Instead, it is what is known as a surrogate protein. This surrogate protein is isolated from sources such as GM E. coli bacteria or, occasionally, a different plant species.[107] This is scientifically unjustifiable because the protein can change as a result of the genetic engineering process and according to the organism within which it is expressed (see 3.1.1 and 3.5.1: StarLink maize). In other words, the same GM gene introduced into a GM plant and into E. coli bacteria can produce proteins that can have very different effects on the people and animals that eat them. In particular, bacteria and plants process newly synthesized proteins in different ways. So even though the amino acid sequences of the two proteins may be identical, their functions can be quite different.

Other reasons why the allergenicity decision tree model is unsatisfactory include:

- A comparison of the new protein in the GM food with the database of known allergens may not detect new allergens.
- Blood serum tests are problematic because allergenic sensitization is an allergen-specific process. So unless the transgenic protein expressed in the GMO is already a common allergen, there is unlikely to be a single sensitized person in the world whose blood serum would react with it.[103]
- Blood serum tests are not useful in detecting uncommon allergens (substances that few people are allergic to).[103]
- A phenomenon known as cross-reactivity can make it difficult to identify from blood serum testing which specific protein out of several is the allergen.[103]
- The artificial stomach tests carried out for regulatory purposes are performed under unrealistic conditions – levels of acidity and digestive enzymes are much higher than would be present in the digestive systems of individuals that would consume the GMO. This makes it likely that the new GM protein will be broken down into fragments that are too small to be potent allergens. In real life, however, the levels of acidity and digestive enzymes in people's stomachs vary, according to age, health status, length of time since they ate their last meal, and other factors. One study found that under the standard conditions used in artificial stomach tests, one of the insecticidal proteins commonly present in GM Bt crops was broken down. But when the researchers adjusted the acidity and enzymes to more realistic levels, the insecticidal protein was highly resistant to being broken down. The authors called for regulatory tests to be carried out in "more physiologically relevant" conditions of lower acidity and lower enzyme levels.[100]

One review concluded that the allergenicity assessment might be useful in assessing GM foods containing a known allergenic protein, but that assessing proteins of unknown allergenicity is "more problematic" and "the predictive value of such an assessment is unknown".[103] A separate review agrees that the standard tests are "not always conclusive", especially when the organism from which the GM gene is taken has no history of dietary use or has unknown allergenicity.[108]

The current allergy assessment system is not reliable because it relies heavily on in vitro tests (test-tube tests on non-living systems, such as the blood serum and artificial stomach tests). But unfortunately, an effective alternative does not yet exist. In vivo tests (tests on living organisms such as animals or humans) are useful for detecting nutritional or toxicological effects of foods, but no animal testing methods have yet been established for allergenicity testing of foods.[103,108,109,110] Independent scientists have asked for such animal tests to be developed.[109,103,108,110]

At present, the only reliable approach to assessing the allergenicity of GMOs would be post-commercialisation monitoring under conditions where consumers are clearly informed when they consume the new GMO and are requested to report any adverse effects to designated authorities. Such post-commercialisation assessments are not required in any country. In countries such as the US and Canada, where consumers are not even informed by labelling of the presence of GMOs in the foods they are eating, the likelihood that allergenicity would be linked to a GMO would be extremely low, unless it caused

acute allergenicity problems to a large portion of the population.

3.7.3. Studies on GM foods confirm existing allergy assessments are inadequate

Studies on GM foods confirm that current allergy assessments are inadequate to detect new allergens created by the genetic engineering process.

In a study on mice fed GM peas containing an insecticidal protein from beans (see 3.1.1), mice showed antibody immune reactions and allergic-type inflammatory responses to the GM protein and chicken egg white protein when it was fed to them with the GM peas.

The mice did not show antibody immune reactions and allergic-type inflammatory responses to beans that naturally contain the insecticidal protein or to egg white protein when it was fed with the natural insecticidal protein obtained from beans. They also did not have an immune response to the egg white protein when it was fed on its own.

These outcomes show that the GM insecticidal protein made the mice more susceptible to developing allergic-type inflammatory reactions to foods eaten with the GM food. This is called immunological cross-priming.

The results indicated that the reaction of the mice to the GM peas was caused by changes brought about by the genetic engineering process. The normally non-immunogenic and non-allergenic insecticidal protein naturally produced in beans was altered in structure and/or function when engineered into peas, becoming a potent immunogen (substance that produces an immune response) and allergen.[4]

It is important to note that this study was not required by regulators, but was carried out as part of the developer's voluntary research programme. The allergenicity of the GM peas would likely not have been spotted by the EU's screening process because the natural, non-GM version of the bean insecticidal protein is not a known allergen. Because of this, blood serum from sensitised individuals would not have been available for regulatory serum tests.

Overall, the study shows that GM foods can contain new allergens and cause new allergic reactions – and that the GMO's allergenicity is unlikely to be detected using the current allergy assessment process.

Two other studies confirm the inadequacy of the current allergy assessment process:

- A study on a commercialised GM insecticidal maize, MON810, showed that the GM plant's proteins were markedly altered compared with those in the non-GM counterpart. Unexpected changes included the appearance of a new form of the protein zein, a known allergen, which was not present in the non-GM maize variety. A number of other proteins were present in both their natural forms and in truncated and lower molecular mass forms.[111] The findings suggest major disruptions in gene structure and function in this GM crop. The EU's allergy assessment failed to pick up these changes and failed to detect the presence of the newly created allergen.
- A GM soy variety modified with a gene from Brazil nuts was found to be capable of producing an allergic reaction in people who are allergic to Brazil nuts. The researchers had genetically engineered the Brazil nut gene into the soy in order to increase its nutritional value. When they tested the effect of this GM soy on blood serum from people allergic to Brazil nuts, they found that the serum produced an allergic response to the soy. Through scratch tests on skin, they confirmed that people allergic to Brazil nuts were allergic to the modified soybean.[64] This study is often cited by GM proponents as evidence of the effectiveness of regulatory processes in identifying allergenic foods before they reach the marketplace. But this is untrue. Tests such as this are not required to be carried out as part of the regulatory assessment of GM foods in any country.

3.7.4. Conclusion

The absence of reliable methods for allergenicity testing and the lack of rigour in current allergy assessments mean that it is impossible to reliably predict whether a GM crop will prove to be allergenic.

3.8 **Myth:** GM animal feed poses no risks to animal or human health **Truth:** GM feed affects the health of animals and may affect the humans who eat their products

Most GM crops go into animal feed. The GM industry and government regulators claim that meat, eggs, and dairy products from GM-fed animals do not need to carry a GM label because GM molecules – DNA and protein – are broken down in the animals' digestive tracts and is not detectable in the final food product.

But this assumption is false. Studies have found:

- GM DNA present in animal feed has been detected in milk sold on the Italian market, though the authors of the study said it was unclear whether the source of the GM DNA was ingestion by the animal or external contamination.[112]
- GM DNA in feed was taken up by the animal's organs and detected in the meat and fish that people eat.[113,114,115,116]
- GM feed was found to affect the health of animals that eat it. GM DNA from soy was detected in the blood, organs, and milk of goats. An enzyme, lactic dehydrogenase, was found at significantly raised levels in the heart, muscle, and kidneys of young goats fed GM soy.[117] This enzyme leaks from damaged cells during immune reactions or injury, so high levels may indicate such problems.
- Bt toxin protein was found circulating in the blood of pregnant women and the blood supply to their foetuses, as well as in the blood of non-pregnant women.[65]
- MicroRNAs (molecules that affect gene expression) of plants have been found in the blood of mammals that have eaten them and were biologically active in those mammals, affecting gene expression and the functioning of important processes in the body. While this study was not carried out on GM plants, it showed that plants that are eaten, including GM plants, could exercise a direct physiological effect on human and animal consumers.[118] The study suggested that the saying, "You are what you eat", may have some scientific credibility.

Given the growing evidence that a diet containing GM crops can damage the health of animals, there could be risks associated with the consumption of products derived from GM-fed animals. We conclude that the argument that meat and dairy products from GM-fed animals do not need to carry a GM label cannot be scientifically justified.

3.9 **Myth:** Genetic engineering will deliver more nutritious crops **Truth:** No GM crop that is more nutritious than its non-GM counterpart has been commercialised and some GMOs are less nutritious

GM proponents have long claimed that genetic engineering will deliver healthier and more nutritious "biofortified" crops. However, no such nutritionally enhanced GM foods are available in the marketplace. In some cases, GM foods have been found to be less nutritious than their non-GM counterparts, due to unexpected effects of the genetic engineering process.

Examples include:

- GM soy had 12–14% lower levels of cancer-fighting isoflavones than non-GM soy.[119]
- Canola (oilseed rape) engineered to contain vitamin A in its oil had much reduced vitamin E and an altered oil-fat composition, compared with the non-GM control.[120]
- Experimental GM rice varieties had unintended major nutritional disturbances compared with non-GM counterparts, although they were grown side-by-side in the same conditions. The structure and texture of the GM rice grain was affected and its nutritional content and value were dramatically altered. The variation ranged from 20 to 74% for amino acids, from 19 to 38% for fatty acids, from 25 to 57% for vitamins, from 20 to 50% for nutritionally important trace elements, and 25% for protein. GM rice varieties variously showed markedly decreased levels of vitamin E, protein, and amino acids. The authors said that their findings "provided alarming information with regard to the nutritional value of transgenic rice" and showed that the GM rice was not substantially equivalent to non-GM.[121]

3.9.1. Golden Rice: More hype than hope?

The best-known attempt to nutritionally improve a GM crop is beta-carotene-enriched "Golden Rice".[122,123] The crop is intended for use in poor countries in the Global South, where vitamin A deficiency causes blindness, illness, and deaths. However, despite over a decade's worth of headlines hyping Golden Rice as a miracle crop, it is still not available in the marketplace.

GM proponents blame excessive regulation and anti-GM activists for delaying the commercialisation of Golden Rice. But the real reasons for the delay seem to be basic research and development problems. The first Golden Rice variety had insufficient beta-carotene content and would have needed to be consumed in kilogram quantities per day to provide the required daily vitamin A intake.[122] As a result, a totally new GM rice variety had to be generated with much higher beta-carotene content.[123]

Also, the process of backcrossing Golden Rice with varieties that perform well in farmers' fields in order to ensure a viable product has taken many years.[124,125] A 2008 article in the journal Science said that there was still a "long way to go" in the backcrossing process.[124]

It has taken over a decade to develop Golden Rice. Yet as of 2012, field trials have not been completed to ensure that it grows successfully in local conditions. Nor has it been tested in toxicological feeding trials on animals to establish whether it is safe to eat. Nevertheless, the rice was fed to human subjects (adults and children) in experiments conducted by researchers at Tufts University, Boston, MA. This was not a safety study but an efficacy test to see whether the human subjects assimilated sufficient beta-carotene and converted it to vitamin A. The efficacy test was conducted without basic toxicological testing having been carried out. This was condemned as a breach of medical ethics and the Nuremberg Code (established after World War II to prevent a repeat of inhumane Nazi experiments on humans) by a group of international scientists in a letter of protest to the Tufts researchers.[126]

In contrast with the problematical Golden Rice, inexpensive and effective methods of combating

vitamin A deficiency have long been available. The most commonly used method is Vitamin A supplements. A review published in the British Medical Journal assessed 43 studies involving 200,000 children and found deaths were cut by 24% if children were given the vitamin. The researchers estimated that giving vitamin A supplements to children under the age of five in developing countries could save 600,000 lives a year. They concluded, "Vitamin A supplements are highly effective and cheap to produce and administer."[127,128]

The World Health Organization's long-standing project to combat vitamin A deficiency uses vitamin A supplements, backed up with education and development programmes. These programmes encourage mothers to breastfeed and teach people how to grow carrots and leafy vegetables in home gardens – two inexpensive, effective, and generally available solutions. WHO says its programme has "averted an estimated 1.25 million deaths since 1998 in 40 countries."[129] According to WHO malnutrition expert Francesco Branca, these approaches are, for now, more promising approaches to combating vitamin A deficiency than Golden Rice.[124]

If the resources that have been poured into developing Golden Rice had been put into such proven programmes, thousands of children and adults could have been saved. The food writer Michael Pollan wrote in an article for the New York Times entitled "The great yellow hype": "These ridiculously obvious, unglamorous, low-tech schemes are being tried today, and according to the aid groups behind them, all they need to work are political will and money."[130]

Pollan is one of several critics who suggested that the real value of Golden Rice lies in its usefulness as a public relations strategy to boost the tarnished image of the biotechnology industry. Pollan wrote that Golden Rice seemed less like a solution to vitamin A deficiency than "to the public-relations problem of an industry that has so far offered consumers precious few reasons to buy what it's selling – and more than a few to avoid it."[130]

3.9.2. Purple cancer-fighting tomato

The John Innes Centre (JIC) in the UK has developed a purple tomato engineered to contain high levels of anthocyanin antioxidants, which have anti-cancer properties. The JIC announced the development of the tomato in 2008 in a press release headlined, "Purple tomatoes may keep cancer at bay".[131] Professor Cathie Martin, who led the research, published an article in the press entitled, "How my purple tomato could save your life".[132]

These claims were based on the results of a preliminary feeding study on cancer-susceptible mice, which found that those fed with the purple tomato had an extended lifespan, measured against control groups fed non-GM tomatoes and a standard rodent diet.[133] Yet as one of the researchers pointed out, the study did not test for possible toxicity, so "We're far from considering a human trial".[134]

Meanwhile, anthocyanins are available in abundance in many common fruits and vegetables, including raspberries, blackberries, blueberries, bilberries, blood oranges, red cabbage, red onions, and aubergine (eggplant).

The JIC's Cathie Martin has argued that tomatoes are consumed by people who might not normally consume many fruits and vegetables, for example, on pizzas and in tomato ketchup on burgers.[132] It is questionable, however, whether people who are conservative in their food choices would eat a tomato that looks, in the words of one journalist, "like a cross between an orange and a black pudding"[135] – let alone a tomato that, at least in Europe, will carry a GM label.

In 2010, a year after the JIC announced its purple GM tomato, Italian researchers announced a non-GM tomato with higher-than-usual levels of the anti-oxidant lycopene.[136] Lycopene, like anthocyanin, has anti-cancer properties.

In 2011 the JIC's GM purple tomato became entirely redundant when Brazilian researchers announced that they had developed a non-GM purple tomato with high levels of anthocyanins and vitamin C.[137] In contrast with the JIC's GM tomato, the non-GM tomatoes received little publicity.

3.9.3. "Biofortified" crops are not a sensible solution to hunger

Most "biofortified" crops, whether produced through GM or conventional breeding, target the

poor and hungry in the Global South and focus on one or two nutrients, such as Vitamin A or iron. Even if we assume that GM can produce more crops with high levels of one or two nutrients, some important topics need to be addressed before concluding that biofortifying crops by whatever means is a sensible approach to malnutrition:

Malnourished people are hungry not because of a lack of biofortified crops, but because they lack money to buy food and, increasingly, access to land on which to grow it. This type of poverty is often due to political conflicts in the country. Another cause is ill-advised "development" programmes that, in return for foreign loans and investment, have forced countries to convert farmland from growing food for people to eat into growing cash crops for export. These are political and economic problems that cannot be solved by offering a biofortified crop, for which the grower will need to be paid. People who have no money to buy basic food will certainly be unable to buy a biofortified food that has taken millions in investment funds to develop.

Malnourished people are not usually deficient in just one or two nutrients, but in many. Focusing on a crop that can deliver one or two nutrients is unhelpful because a balance of nutrients is needed for proper absorption. For example, in order to absorb vitamin A, people need to have enough fat in their diet. This problem would need to be addressed before they could benefit from vitamin A-enriched food.

Manipulating nutrients in food is controversial because it can be viewed as medicating food. Dosage is difficult to control and certain nutrients may be needed by one person, yet be excessive and potentially dangerous for the next. Also, nutritional theory is a fast-moving discipline, with today's desirable nutrient becoming tomorrow's undesirable contaminant.[138]

3.9.4. Non-GM biofortified crops are already available

If we assume that biofortified foods are a desirable approach to malnutrition, plenty of non-GM crop varieties are available now that do not present the risks and uncertainties of genetic engineering (see Section 7).

In addition, there are ways of adding nutrients to people's diets that do not involve the considerable expense of crop breeding. These include a rice fortified with iron and vitamins, which has been reported in a preliminary study to have caused dramatic falls in anaemia and vitamin B1 deficiency in children.[139]

Conclusion to Section 3

Contrary to frequent claims that there is no evidence of dangers to health from GM foods and crops, peer-reviewed studies have found harmful effects on the health of laboratory and livestock animals fed GMOs. Effects include toxic and allergenic effects and altered nutritional value.

Most animal feeding studies on GMOs have only been medium-term in length (30–90 days). While GM proponents claim that the observed harmful effects on health are not "biologically relevant" or "adverse", such claims are scientifically unjustifiable; these terms have not even been properly defined.

What is needed are long-term and multi-generational studies on GMOs to see if the changes found in medium-term studies, which are suggestive of harmful health effects, will develop into serious disease, premature death, or reproductive or developmental effects. Today, such studies are not required by regulators anywhere in the world.

Moreover, the system for assessing the allergenic potential of GM foods in place in the EU today – although it is probably the most rigorous of any assessment system anywhere in the world – is inadequate and unlikely to identify new allergens.

While GM proponents claim that GM can provide nutritionally enhanced (biofortified) foods, no such GM foods are available on the market.

The most widely publicised example of a GM nutritionally enhanced food, Golden Rice, has used up millions of dollars' worth of research and development money. Yet it has not undergone

proper toxicological testing and, after more than a decade, is still not ready for the market. In contrast, tried, tested, and inexpensive means of preventing and curing vitamin A deficiency are successful when applied but are under-utilised due to underfunding.

Aspirational claims of nutritionally enhanced GM crops are a dangerous distraction from the real causes of hunger, which are poverty and a lack of access to land on which to grow food. But if society decides that nutritionally enhanced foods are an important route to food security, it need not wait for expensive GM "solutions". Conventional plant breeding has already successfully and safely produced many such biofortified foods.

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4. HEALTH HAZARDS OF ROUNDUP & GLYPHOSATE

Over 75% of all GM crops are engineered to tolerate herbicides. Roundup Ready (RR) soy is the most widely grown GM crop, making up 52% of all GM crops.[1] RR soy is engineered to tolerate Roundup herbicide, the main ingredient of which is glyphosate. The RR gene enables farmers to spray the field liberally with herbicide. All plant life is killed except the crop.

The widespread adoption of GM RR soy in North and South America has led to massive increases in the use of Roundup and other glyphosate herbicides.[2]

In South America, a public health crisis has emerged around the spraying of Roundup on GM soy, which is often carried out from the air. The problem made headlines on the publication of a 2010 study by Argentine researchers showing that glyphosate and Roundup caused malformations (birth defects) in frog and chicken embryos at doses far lower than those used in agricultural spraying. The malformations seen in the experimental embryos were similar to human birth defects reported in GM soy-growing areas of South America.

The researchers said the results were relevant to humans because humans have the same developmental mechanisms as frogs and chickens. The study identified the pathway through which glyphosate and Roundup affect embryonic development, the retinoic acid signalling pathway.[3]

A report by physicians in Argentina based on clinical data reported the following health effects in people exposed to spraying of agrochemicals (mostly glyphosate) on GM Roundup Ready soy: increased incidence of birth defects, miscarriages, infertility, cancers, DNA damage (which can lead to cancer and birth defects), neurological developmental problems in children, kidney failure, respiratory problems, and allergies.[4]

A report commissioned by the provincial government of Chaco, Argentina, found that the rate of birth defects increased fourfold and rates of childhood cancers tripled in only a decade in areas where rice and GM soy crops are heavily sprayed. The report noted that problems centred on "transgenic crops, which require aerial and ground spraying with agrochemicals"; glyphosate was named as a chemical of concern.[5]

These issues are relevant not only to people living in regions where GM RR crops are grown, but for consumers who eat products made from crops sprayed with glyphosate. GM RR crops do not break down glyphosate, but absorb it. Some is broken down (metabolised) into a substance called aminomethylphosphonic acid (AMPA). Both glyphosate and AMPA remain in the plant and are eaten by people and animals. Both are toxic.

Scientific evidence suggests that Roundup and other commercial formulations are more toxic than glyphosate alone – yet it was glyphosate alone that was tested by industry prior to market authorization and approved by regulators. The herbicide formulations as they are sold and used have not been properly tested and assessed for safety.

Section at a glance

- Roundup, the herbicide that most GM crops are engineered to tolerate, based on the chemical glyphosate, is marketed as a "safe" herbicide, based on outdated and largely unpublished studies by manufacturers.
- But laboratory and epidemiological studies confirm that Roundup poses serious health hazards, including endocrine (hormone) disruption, DNA damage, cancer, birth defects, and neurological disorders.
- Some of these effects are found at low, realistic doses that could be found as residues in food and feed crops and in contaminated water. People who eat foods made from GM crops could be ingesting potentially dangerous levels of Roundup residues.
- Roundup and glyphosate have been detected in air, rain, groundwater, in people's urine, and even circulating in women's blood. Glyphosate can cross the placental barrier and the unborn foetus could thus be exposed.
- The "safe" dose for Roundup exposure set by regulators is not based on up-to-date objective evidence; thus current regulations do not protect the public.

4.1 **Myth:** Roundup is a safe herbicide with low toxicity **Truth:** Roundup poses major health hazards

Roundup is marketed as a "safe" herbicide, based on outdated and largely unpublished studies by manufacturers.[6] But independent toxicological and epidemiological studies confirm that Roundup and glyphosate pose serious health hazards, as detailed below.

4.1.2. People who eat Roundup Ready crops may be eating toxic residues

The effects on animals and humans of eating increased amounts of glyphosate herbicide residues on such crops have not been properly investigated. On the contrary, regulators have ignored risks and changed safety rules to allow higher levels of glyphosate residues into the food and feed chain.

For example, after the 1996 commercialisation of GM RR soy, EU regulators raised the allowed maximum residue limit (MRL) for glyphosate in imported soy 200-fold, from 0.1 mg/kg to 20 mg/kg.[7] The UK government claimed that the move was necessary to accommodate the new farm practice of using glyphosate as a desiccant to "burn down" crops before harvest, making grains or beans easier to gather.[8] But it also conveniently coincided with the introduction of RR soy.

Indeed, a 1994 report of the Joint FAO/WHO Meetings on Pesticide Residues (JMPR) indirectly admitted that GM soy was a factor in the need for the higher limit. This JMPR meeting appears to have been the source of the recommendation for the new higher residue limit. In its report, the JMPR recommended the higher limit of 20 mg/kg for soybeans. The JMPR said the change was needed because of a combination of two factors: glyphosate's use as a desiccant before harvest; and to accommodate "sequential application of glyphosate in the crop"[9] – a practice that is only possible with GM RR soy, as it would kill non-GM soy.

In a 1999 press interview, Malcolm Kane, the then recently-retired head of food safety at UK supermarket chain Sainsbury's, confirmed that the European regulators raised the residue limit to "satisfy the GM companies" and smooth the path for GM soy to enter the food and feed market. Kane added, "One does not need to be an activist or overtly anti-GM to point out that herbicide-resistant crops come at the price of containing significant chemical residues of the active chemical in the commercial weedkiller."[8]

This high residue limit is potentially unsafe, based on data from independent studies that EU regulators ignored in setting their claimed safe daily dose.[10,11,12] Glyphosate, AMPA, and especially the commercial formulation Roundup have been found to be toxic, in some cases at extremely low levels.[13,14,15] Roundup damages and kills human cells at levels below those used in agriculture[16] and at residual levels to be expected in food and feed derived from Roundup-treated crops.[13] Roundup is a potent endocrine disruptor (disturbs hormone function) at concentrations up to 800 times lower than the highest permitted levels in food and feed.[17] So people who eat food products from GM RR crops are eating amounts of these substances that may have toxic effects.

4.1.3. Studies show toxic effects of glyphosate and Roundup

Independent studies on human cells and experimental animals have shown that glyphosate and Roundup have serious toxic effects, in many cases at low levels that could be found in the environment or as residues in food or feed.[13,14,15] The added ingredients (adjuvants) in Roundup are themselves toxic and increase the toxicity of glyphosate by enabling it to penetrate human and animal cells more easily.[13,18,19] Findings include:

- Glyphosate and Roundup caused malformations in frog and chicken embryos.[3]
- Roundup caused skeletal malformations in rat foetuses.[20]
- Industry's own studies conducted for regulatory purposes as long ago as the 1980s show that glyphosate caused birth defects in rats and rabbits. These effects were seen not only at high, maternally toxic doses, but also

at lower doses. Interestingly, these effects were discounted by regulators, who approved glyphosate for use in food production.[10]

- Roundup caused liver and kidney toxicity in fish at sublethal doses. Effects in the liver included haemorrhage and necrosis (death of cells and living tissue).[21]
- Roundup caused total cell death in human cells within 24 hours at concentrations far below those used in agriculture and corresponding to levels of residues found in food and feed.[13]
- Roundup caused death of human cells and programmed cell death at a concentration of 50 parts per million, far below agricultural dilutions.[16]
- Roundup was a potent endocrine disruptor at levels up to 800 times lower than residue levels allowed in food and feed. It was toxic to human cells and caused DNA damage at doses far below those used in agriculture.[17]
- Glyphosate was toxic to human placental cells and is an endocrine disruptor in concentrations lower than those found with agricultural use. Roundup adjuvants amplified glyphosate's toxicity by enabling it to penetrate cells more easily and to bioaccumulate in cells.[15]
- Glyphosate and Roundup damaged human embryonic and placental cells at concentrations below those used in agriculture, suggesting that they may interfere with human reproduction and embryonic development.[14]
- Glyphosate's main metabolite (environmental breakdown product), AMPA, altered cell cycle checkpoints by interfering with the cells' DNA repair machinery.[22,23,19,24] The failure of cell cycle checkpoints is known to lead to genomic instability and cancer in humans.
- Glyphosate and AMPA irreversibly damaged DNA, suggesting that they may increase the risk of cancer.[25,26]
- Glyphosate promoted cancer in the skin of mice.[27]
- Roundup caused cell and DNA damage to epithelial cells derived from the inside of the mouth and throat, and glyphosate alone caused DNA damage, raising concerns over the safety of inhaling the herbicide, one of the most common ways in which people are exposed. Importantly, both glyphosate and Roundup caused DNA damage at concentrations below those required to induce cell damage, suggesting that the DNA damage was caused directly by glyphosate and Roundup instead of being an indirect result of cell toxicity.[28]

4.1.4. Epidemiological studies on Roundup show links with serious health problems

Epidemiological studies show a link between Roundup/glyphosate exposure and serious health problems, including:

- DNA damage[27]
- Premature births and miscarriages[28,29]
- Birth defects including neural tube defects and anencephaly (absence of a large part of the brain and skull)[32,33]
- Multiple myeloma, a type of cancer[34]
- Non-Hodgkin's lymphoma, a type of cancer[35,36,37]
- Disruption of neurobehavioral development in children of pesticide applicators – in particular, attention-deficit disorder (ADD) and attention-deficit hyperactivity disorder (ADHD).[38]

Epidemiological studies cannot prove a cause-and-effect relationship between exposure to a suspect substance and a health effect. However, in the case of glyphosate/Roundup, toxicological studies carried out under controlled laboratory conditions confirm the causal relationship to health problems (see 4.1.3).

4.1.5. People are widely exposed to glyphosate

Glyphosate-based herbicides are widely used outside of the farm environment – for example, by municipal authorities to control weeds on roadsides and in parks and school grounds, as well as by home gardeners. So even when farm use is excluded, people's exposure to glyphosate is significant. In agricultural areas where GM glyphosate-resistant crops are grown, exposure is likely to increase exponentially.

Study findings on human exposures and body burdens include:

- Glyphosate was detected in between 60 and

100% of air and rain samples taken in the American Midwest during the crop growing season.[39] Roundup Ready GM crops are widely planted in this region.

- Glyphosate and its main breakdown product, AMPA, were frequently detected in streams in the American Midwest during the growing season.[40]
- Glyphosate and its main breakdown product AMPA were washed out of the root zone of clay soils in concentrations that exceeded the acceptable quantities for drinking water (0.1 µg/l), with maximum values of over 5 µg/l.[41]
- Glyphosate was found circulating in the blood of non-pregnant women, albeit at low levels.[42]
- Urinary body burdens of glyphosate in farm and non-farm families in Iowa were over 900 parts per billion (0.9 mg per kg of body weight) in 75% of farmers, 67% of wives, and 81% of farmers' children. Urinary burdens in non-farm children were slightly higher than those in farm children. The authors suggested that this was because of the widespread use of glyphosate in non-farm areas, such as in people's gardens.[43]

The placental barrier in mammals is often claimed to protect the unborn foetus from glyphosate exposures. But this claim was shown to be false by a research study modeling human exposures, in which 15% of administered glyphosate crossed the human placental barrier and entered the foetal compartment.[44]

4.1.6. People are not protected by the current regulations on glyphosate

An analysis of glyphosate's current approval in the EU and in the US suggests that the "acceptable daily intake" (ADI) level, the level of exposure that is deemed safe for humans over a long period of time, is inaccurate and potentially dangerously high.[10]

Regulators calculate the ADI on the basis of industry studies submitted to the regulators in support of the chemical's approval. The figure used to set the ADI is the highest dose at which no adverse effect is found (the No Observed Adverse Effect Level or NOAEL), which is also lower than the lowest dose that has a toxic effect (the Lowest Observed Adverse Effect Level or LOAEL). The ADI is derived by dividing this figure by 100, to allow a safety margin.

The current ADI for glyphosate is 0.3 mg per kg of body weight per day (written as 0.3 mg/kg bw/d).

But this ADI has been shown to be inaccurate by two independent studies on Roundup using an animal (rat) and exposure route (oral feeding) approved by EU and international regulators. The studies found that:

- Roundup was a potent endocrine disruptor and caused disturbances in the reproductive development of rats when the exposure was performed during the puberty period. Adverse effects, including delayed puberty and reduced testosterone production, were found at all dose levels, including the LOAEL of 5 mg/kg bw/d.[11]
- Glyphosate herbicide caused damage to rats' liver cells that the researchers said was probably "irreversible" at a dose of just 4.87 mg/kg bw/d.[12]

These studies did not find a safe or "no effect" level (NOAEL). Even the lowest dose tested produced a toxic effect and no further experiments were done with lower doses to establish the NOAEL. A reasonable estimate of the NOAEL might be 2.5 mg/kg of body weight (though this estimate should, of course, be tested). Then, applying the 100-fold safety factor, the ADI should be 0.025 mg/kg bw/d – 12 times lower than the one currently in force.

Even if only the industry studies are considered, the current ADI should still be lower. An objective analysis of these studies results in a more objectively accurate ADI of 0.1 mg/kg bw/d, one-third of the current ADI.[10]

4.1.7. Arguments that Roundup replaces more toxic herbicides are false

GM proponents often argue that Roundup has replaced more toxic herbicides and that GM RR crops therefore reduce the toxic burden on humans and the environment. But this is false. GM RR crops have not only increased the use of glyphosate herbicides but have also increased

the use of other, potentially even more toxic herbicides, due to the spread of glyphosate-resistant weeds (see Section 5). And as we have seen, the presumed safety of Roundup owes more to clever marketing than to objective scientific findings.

Conclusion to Section 4

GM Roundup Ready (RR) soy is the most widely grown GM crop. It is engineered to tolerate being sprayed with Roundup herbicide, based on the chemical glyphosate. Widespread planting of GM soy in North and South America has led to large increases in the amount of glyphosate herbicide used. Regulators have responded by raising the allowed residue limit of glyphosate in crops eaten by people and animals. So people and animals that eat GM RR crops are eating potentially toxic herbicide residues.

Regulators and industry claim that this is safe because Roundup has low toxicity. But these claims – as well as the supposed "safe" level of glyphosate set by regulators – are based on outdated industry studies, the findings of which have been thrown into question by numerous independent studies. These studies show that Roundup and glyphosate are not safe but pose serious health risks. Effects found in animal studies and test-tube studies on human cells include cell death and damage, damage to DNA, disruption of hormones, birth defects, and cancer. Some of these effects have been found at levels far below those used in agriculture and corresponding to low levels of residues in food and feed. The added ingredients in Roundup (adjuvants) increase the toxicity of glyphosate, and the main breakdown product of glyphosate, AMPA, is also toxic.

Effects of exposure to glyphosate herbicides on humans found in epidemiological studies include DNA damage, premature birth and miscarriage, cancer, and attention deficit disorder in children.

The widespread use of glyphosate herbicides – not just on farms but in gardens, on roadsides, and in parks and school grounds – means that many people are exposed. In addition, glyphosate does not stay where it is applied but moves around the environment. It is frequently found in rain, air, streams, and groundwater, and even in women's blood.

GM crops have increased the use of glyphosate and thus people's exposure to it, presenting a risk that has not been adequately considered in regulatory assessments of GM crops.

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5. GM CROPS – IMPACTS ON THE FARM AND ENVIRONMENT

Section at a glance

- GM does not increase intrinsic yield. Some GM crops have lower yields than non-GM counterparts.
- GM crops have increased pesticide use by 383 million pounds in the US in the first 13 years since their introduction.
- The modest reduction in chemical insecticide sprays from GM Bt insecticidal crops is swamped by the large increase in herbicide use with GM herbicide-tolerant crops.
- GM herbicide-tolerant crops have caused an over-reliance on a single herbicide, glyphosate, leading to the emergence of resistant superweeds and causing farmers to use more herbicides, including older toxic ones like dicamba and 2,4-D.
- The GM companies' solution to the glyphosate-resistant superweeds problem is stacked trait GM crops that tolerate applications of multiple herbicides – and mixtures of herbicides. Weed scientists warn that this will cause herbicide use to triple, foster multi-herbicide-resistant superweeds, and undermine sustainable farming.
- Claims of environmental benefits from no-till of farming as used with GM herbicide-tolerant crops collapse once herbicide use is taken into account.
- GM Bt crops do not eliminate insecticide use – they merely change the way in which insecticides are used. The plant itself becomes an insecticide.
- GM Bt technology is being undermined by the spread of insect pests that are resistant to Bt crops, forcing farmers to use chemical insecticides as well as buying expensive Bt seed.
- Bt toxins in GM Bt crops are not specific to insect pests, but harm beneficial insect pest predators and soil organisms.
- Roundup used on GM herbicide-tolerant crops is not environmentally safe. It persists in the environment and has toxic effects on wildlife as well as humans (section 4).
- Roundup increases plant diseases, notably Fusarium, a fungus that causes sudden death and wilt in soy plants and is toxic to humans and livestock.
- The economic impacts on farmers of adopting GM crops were described in a study for the US Dept of Agriculture as "mixed or even negative".
- "Coexistence" between GM and non-GM crops is impossible as non-GM and organic crops become contaminated, resulting in lost markets and massive economic losses.
- The possibility that GM traits could spread not only to related species by cross-pollination but also to unrelated species by horizontal gene transfer, should be investigated before commercialising GM crops.

> *"Over the past decade, corporate and government managers have spent millions trying to convince farmers and other citizens of the benefits of genetically modified (GM) crops. But this huge public relations effort has failed to obscure the truth: GM crops do not deliver the promised benefits; they create numerous problems, costs, and risks; and ... consumers and foreign customers alike do not want these crops.*
>
> *"It would be too generous even to call GM crops a solution in search of a problem: These crops have failed to provide significant solutions, and their use is creating problems – agronomic, environmental, economic, social, and (potentially) human health problems."*
>
> – National Farmers Union of Canada[1]

GM crops are promoted on the claimed basis that they give higher yields, reduce pesticide use, and benefit farmers and the environment. But independent studies either contradict these claims or show them to be inflated. GM crop technology is already failing under the onslaught of developments such as the spread of herbicide-resistant superweeds and pests resistant to the Bt toxin engineered into crops. These failures mean increasing costs to farmers and harm to the environment.

On-farm and environmental impacts of GM crops are not limited to the effects of the GM crop itself – for example, GM genes can spread to non-GM and organic crops. They also include the effects of the pesticide that the crop is engineered to contain or that it is designed to be grown with. Research shows that impacts are occurring from all these sources.

Some of these impacts occur with industrially-grown non-GM crops, too. But often, GM proponents obscure the negative effects of GM crops by comparing them with crops grown under chemically-based agricultural systems. They then draw the conclusion that GM crops have less harmful impacts.

But this is to compare one unsustainable agricultural system with another. A more meaningful comparison, and one that would help advance agricultural technology, would be to compare GM with agroecological or integrated pest management (IPM) systems. Many farmers outside the certified organic sector already use agroecological and IPM methods. This progressive trend should be encouraged. Instead, it is being delayed by the false hope offered farmers by GM crops. In contrast to agroecological methods, GM agriculture is an extension of chemically-based, high-input agriculture.

Below, we point out some of the flaws in the common arguments used to promote GM crops.

5.1 **Myth:** GM crops increase yield potential **Truth:** GM crops do not increase yield potential – and in many cases decrease it

GM crops are often claimed to give higher yields than naturally bred varieties. But the data do not support this claim. At best, GM crops have performed no better than their non-GM counterparts, with GM soybeans giving consistently lower yields.[3]

Controlled field trials comparing GM and non-GM soy production suggested that 50% of the drop in yield is due to the disruption in genes caused by the GM transformation process.[4] Similarly, field tests of Bt maize hybrids showed that they took longer to reach maturity and produced up to 12% lower yields than their non-GM counterparts.[5]

A US Department of Agriculture report confirmed the poor yield performance of GM crops, saying, "GE [genetically engineered] crops available for commercial use do not increase the yield potential of a variety. In fact, yield may even decrease.... Perhaps the biggest issue raised by these results is how to explain the rapid adoption of GE crops when farm financial impacts appear to be mixed or even negative."[6]

The definitive study to date on GM crops and yield is *Failure to Yield*,[2] by Dr Doug Gurian-Sherman, senior scientist at the Union of Concerned Scientists and former biotech adviser to the US Environmental Protection Agency. The study, which is based on peer-reviewed research and official US Department of Agriculture (USDA) data, was the first to tease out the contribution of genetic engineering to yield performance from the gains made through conventional breeding. It is important to bear in mind when evaluating the yield performance of GM crops that biotech companies insert their proprietary GM genes into the best-performing conventionally bred varieties.

The study also differentiated between intrinsic and operational yield. Intrinsic or potential yield, the highest that can be achieved, is obtained when crops are grown under ideal conditions. In contrast, operational yield is obtained under field conditions, when environmental factors such as pests and stress result in yields that are considerably less than ideal. Genes that improve operational yield reduce losses from such factors.

> *"Commercial GE crops have made no inroads so far into raising the intrinsic or potential yield of any crop. By contrast, traditional breeding has been spectacularly successful in this regard; it can be solely credited with the intrinsic yield increases in the United States and other parts of the world that characterized the agriculture of the twentieth century."*
> – Doug Gurian-Sherman, former biotech advisor to the US Environmental Protection Agency (EPA) and senior scientist at the Union of Concerned Scientists in Washington, DC[2]

The study found that GM technology has not raised the intrinsic yield of any crop. The intrinsic yields of corn and soybeans did rise during the twentieth century, but this was not as a result of GM traits, but due to improvements brought about through traditional breeding.

The study found that GM soybeans did not increase operational yields, either. GM maize increased operational yields only slightly, mostly in cases of heavy infestation with European corn borer. Bt maize offered little or no advantage when infestation with European corn borer was low to moderate, even when compared with conventional maize that was not treated with insecticides.

The study concluded, "Commercial GE crops have made no inroads so far into raising the intrinsic or potential yield of any crop. By contrast, traditional breeding has been spectacularly successful in this regard; it can be solely credited with the intrinsic yield increases in the United States and other parts of the world that characterized the agriculture of the twentieth century."[2]

In 2009, in an apparent attempt to counter criticisms of low yields from its GM soy, GM seed producer Monsanto released its new generation of

supposedly high-yielding GM soybeans, RR2 Yield. But a study carried out in five US states involving 20 farm managers who planted RR2 soybeans in 2009 concluded that the new varieties "didn't meet their [yield] expectations".[7] In June 2010 the state of West Virginia launched an investigation of Monsanto for false advertising claims that RR2 soybeans gave higher yields.[8]

If GM cannot increase yields even in the United States, where high-input, irrigated, heavily subsidised commodity farming is the norm, it is irresponsible to assume that it would improve yields in the Global South, where farmers may literally bet their farms and livelihoods on a crop.

We agree with the conclusion of *Failure to Yield* that the funding and research that are currently poured into trying to produce high-yield GM crops should be redirected toward approaches that are proven effective in improving crop yields, including conventional plant breeding as well as use of agroecological practices. These are by far the most efficient, affordable, and widely practised methods of improving yield.

5.2 **Myth:** GM crops decrease pesticide use **Truth:** GM crops increase pesticide use

> *"GE crops have been responsible for an increase of 383 million pounds of herbicide use in the US over the first 13 years of commercial use of GE crops (1996–2008). This dramatic increase in the volume of herbicides applied swamps the decrease in insecticide use attributable to GE corn and cotton, making the overall chemical footprint of today's GE crops decidedly negative... The primary cause of the increase [is] the emergence of herbicide-resistant weeds."*
> – Dr Charles Benbrook, agronomist[9]

> *"The promise was that you could use less chemicals and produce a greater yield. But let me tell you none of this is true."*
> – Bill Christison, president of the US National Family Farm Coalition[10]

GM crops are claimed by proponents to reduce pesticide use (the term "pesticide" includes herbicides, which technically are pesticides). But this is untrue. Herbicide-tolerant crops have been developed by agrochemical firms specifically to depend upon agrochemicals and have extended the market for these chemicals. Far from weaning agriculture away from environmentally damaging chemicals, GM technology has prolonged and extended the chemically-based agricultural model.

The adoption of GM Roundup Ready crops, especially soy, has caused massive increases in the use of glyphosate worldwide.[9,11,12,13,14]

A report by agronomist Dr Charles Benbrook using official US Department of Agriculture data looked at the effects on pesticide use of the first thirteen years of GM crop cultivation in the United States, from 1996 to 2008.[9] Crops taken into account were GM herbicide-tolerant and GM Bt maize varieties, GM Roundup Ready soy, and GM herbicide-tolerant and GM Bt cotton varieties.

The report found that Bt maize and cotton delivered reductions in chemical insecticide use totalling 64.2 million pounds (29.2 million kg) over the thirteen years – though even the sustainability of this trend is questionable, given the emergence of Bt-resistant pests and the changes in insecticide use patterns (see 5.3, below).

But herbicide-tolerant maize, soy, and cotton caused farmers to spray 383 million more pounds (174 million kg) of herbicides than they would have done in the absence of herbicide-tolerant seeds. This massive increase in herbicide use swamped the modest 64.2 million pound reduction in chemical insecticide use attributed to Bt maize and cotton.

The report showed that recently, herbicide use on GM fields has veered sharply upward. Crop years 2007 and 2008 accounted for 46% of the increase in herbicide use over thirteen years across the three herbicide-tolerant crops. Herbicide use on GM herbicide-tolerant crops rose 31.4% from 2007 to 2008.

The report concluded that farmers applied 318 million more pounds of pesticides as a result of planting GM seeds over the first thirteen years of commercial use. In 2008, GM crop fields required over 26% more pounds of pesticides per acre (1 acre = 0.4 hectares) than fields planted to non-GM varieties.

The report identified the main cause of the increase in herbicide use as the spread of glyphosate-resistant weeds.

5.2.1. Glyphosate-resistant superweeds

The widespread use of Roundup Ready crops has led to over-reliance on a single herbicide – glyphosate, commonly sold as Roundup. This has resulted in the rapid spread of glyphosate-resistant weeds in countries where GM crops are planted.[15] Resistant weeds include pigweed,[16] ryegrass,[17] and marestail.[18]

The Herbicide Resistance Action Committee (HRAC), financed by the pesticide industry, lists 21 glyphosate-resistant weeds around the world. In the United States, glyphosate-resistant weeds have been identified in 22 states.[19]

When resistant weeds first appear, farmers

often use more glyphosate herbicide to try to control them. But as time passes, no amount of glyphosate herbicide is effective and farmers are forced to resort to potentially even more toxic herbicides, such as 2,4-D, and mixtures of herbicides.[15,16,17,18,20,21,22,23,24,25,26]

US farmers are going back to more labour-intensive methods like ploughing – and even pulling weeds by hand.[25] In Georgia, tens of thousands of acres of farmland have been abandoned after being overrun by glyphosate-resistant pigweed.[27,28]

An article in Monsanto's hometown newspaper, the St Louis Post-Dispatch, said of the Roundup Ready system, "this silver bullet of American agriculture is beginning to miss its mark."[29] As glyphosate-resistant weeds undermine the Roundup Ready farming model, Monsanto has taken the extraordinary step of subsidizing farmers' purchases of competing herbicides to supplement Roundup.[25,30]

5.2.2. How are superweeds created?

Many glyphosate-resistant weeds appear through what is known as selection pressure – only those weeds that survive being sprayed with glyphosate herbicides pass on their genes, leading to a steady increase in glyphosate-resistant plants in the weed population.

But there is a second route through which glyphosate-resistant weeds develop: GM crops can pass on their genes for herbicide tolerance to wild or cultivated non-GM relatives. GM canola has been found to pass on its glyphosate-tolerance genes to related wild plants such as wild mustard, turning them into difficult-to-control superweeds. The GM herbicide-tolerance gene was shown to persist in these weed populations over a period of six years.[31]

GM canola itself has also become a weed. Feral canola populations have acquired resistance to all of the main herbicides used in Canada,[24] making it difficult and expensive to control "volunteer" canola in soy and maize fields. Feral herbicide-resistant canola has also become a problem in sugar beet fields in the US, where canola seeds are reported to be deposited by defecation from geese migrating from Canada.[32]

5.2.3. GM industry "solution" to superweeds: More herbicides

The industry's solution to the glyphosate-tolerant superweeds crisis has been first, to aggressively market pre-mix herbicide products to farmers, and second, to develop "stacked trait" crop varieties resistant to multiple herbicides. These stacked trait crops enable farmers to spray mixtures of weedkillers freely, instead of having to apply them carefully in order to spare crops.[26] Simple arithmetic indicates that this will double or triple the amount of herbicide applied to a given field.

Dow has applied to release a multi-herbicide-tolerant soybean, engineered to tolerate being sprayed with glyphosate, glufosinate, and 2,4-D[34] – an ingredient of the defoliant Agent Orange. In 2012 Dow sparked public outrage when it applied to the US Department of Agriculture to commercialise its 2,4-D-tolerant corn.[35]

Weed scientists warn that such multi-herbicide-tolerant crops will cause an increase in 2,4-D use, trigger an outbreak of still more intractable weeds resistant to both glyphosate and 2,4-D, and undermine sustainable approaches to weed management.[33]

In fact, weed species that are resistant to dicamba,[36] to 2,4-D,[37] and to multiple herbicides[38] already exist.

Most stacked-trait superweeds emerge through what is known as selection pressure, where only those weeds that can tolerate herbicide survive to pass on their genes.

But there is another route through which superweeds can emerge: cross-pollination of GM herbicide-tolerant crops within the crop species or with wild relatives. "Stacked trait" multi-herbicide-resistant oilseed rape (canola) plants have already appeared as a result of accidental cross-pollination between GM crops engineered to tolerate different herbicides. As early as 1998, oilseed rape plants were found that tolerated up to three different herbicides.[39]

A Canadian government study showed that after just 4–5 years of commercial growing, GM oilseed rape engineered to tolerate different single herbicides had cross-pollinated to create stacked trait plants resistant to up to three broad-spectrum herbicides, posing a serious problem for farmers.[22,23,24]

5.2.4. Conclusion

GM herbicide-tolerant crops have led to massive increases in herbicide use and a resulting spread of herbicide-resistant weeds. Farmers have to resort to spraying more herbicide, or mixtures of herbicides, to try to control weeds. This "chemical treadmill" model of farming is especially impractical for farmers in the Global South, who cannot afford to buy more or different herbicides in an effort to control resistant weeds.

5.3 **Myth:** No-till farming with GM crops is environmentally friendly **Truth:** Claims of environmental benefits from GM no-till farming are unsound

GM proponents claim that GM herbicide-tolerant crops, especially GM Roundup Ready (RR) soy, are environmentally friendly because they allow farmers to adopt the no-till system of cultivation. No-till farming avoids ploughing in order to conserve soil and water, and supposedly to reduce carbon dioxide emissions. In no-till cultivation of GM Roundup Ready soy, weeds are controlled through herbicide applications rather than mechanically, through ploughing.

There are at least two problems with this argument:

- No-till or low-till farming can be – and is – practised in chemically-based and agroecological farming. Farmers do not have to adopt GM crops or use herbicides to practise no-till.
- Claims of environmental benefits for GM crops with no-till cultivation have been shown to be misleading. One study compared the environmental impacts of growing GM RR and non-GM soy, using an indicator called Environmental Impact Quotient (EIQ). EIQ assesses the negative environmental impacts of the use of pesticides and herbicides on farm workers, consumers and ecology (fish, birds, bees and other beneficial insects). The study found that in Argentina, the negative environmental impact of GM soy was higher than that of non-GM soy in both no-till and tillage systems because of the herbicides used. Also, the adoption of no-till raised the EIQ, whether the soy was GM RR or non-GM. The main reason for the increase in herbicides used in no-till systems was the spread of glyphosate-resistant superweeds.[40]

We conclude that claims of environmental benefits from no-till farming with GM crops are unjustified.

Herbicide-tolerant crops undermine sustainable agriculture

"Agricultural weed management has become entrenched in a single tactic – herbicide-resistant crops – and needs greater emphasis on integrated practices that are sustainable over the long term. In response to the outbreak of glyphosate-resistant weeds, the seed and agrichemical industries are developing crops that are genetically modified to have combined resistance to glyphosate and synthetic auxin herbicides. This technology will allow these herbicides to be used over vastly expanded areas and will likely create three interrelated challenges for sustainable weed management. First, crops with stacked herbicide resistance are likely to increase the severity of resistant weeds. Second, these crops will facilitate a significant increase in herbicide use, with potential negative consequences for environmental quality. Finally, the short-term fix provided by the new traits will encourage continued neglect of public research and extension in integrated weed management."

– Mortensen DA, et al. Navigating a critical juncture for sustainable weed management. BioScience 2012; 62: 75-84[33]

5.4 **Myth:** GM Bt crops reduce insecticide use **Truth:** GM Bt crops merely change the way in which insecticides are used

GM proponents claim that GM Bt crops reduce insecticide use, as farmers do not have to spray chemical insecticides. But this claim does not stand up to analysis, since the Bt gene turns the plant itself into an insecticide and because pest adaptation makes the GM pesticide less effective over time, making it necessary for farmers to revert to the use of chemical pesticides after just a few years. The genetically modified insecticide is present in active form in every part of the crop, including the parts that people and animals eat.

So Bt crops do not reduce or eliminate insecticides. They temporarily change the type of insecticide and the way in which it is used – from sprayed on, to built in. But in the long term, use of chemical pesticides must be resumed, as long as the industrial agricultural model is followed.

Even if we choose to ignore this factor and only consider the temporary reduction in chemical insecticide sprays due to Bt crops, the figure is unspectacular (see 5.2, above) – a reduction of 64.2 million pounds (29.2 million kg) over the first thirteen years of GM crop cultivation in the United States. This reduction is swamped by the massive increase in pesticide use resulting from the adoption of GM herbicide-tolerant crops, which has caused farmers to spray 383 million more pounds (174 million kg) of herbicides than they would have done in the absence of GM herbicide-tolerant seeds (herbicides are technically pesticides).[9]

Even the modest reduction in chemical insecticides attributed to GM Bt crops is proving unsustainable, due to the emergence of pests resistant to Bt toxin and secondary pests, as explained below. Moreover, there is a question mark over whether Bt crops can truly be said to have reduced chemical insecticide use in view of changes in the types of insecticides used and in the methods of application.

5.4.1. Resistant pests are making Bt technology redundant

GM Bt insecticidal crops express the Bt toxin in every cell for their entire lifetime, constantly exposing pests to the toxin. This is different from the traditional use of natural Bt as a spray, where the targeted pests are only exposed for a brief period before the Bt breaks down in daylight. Exposing pests to a pesticide for long periods of time inevitably speeds up the emergence of resistant pests, since selective pressure eliminates all but the most resistant pests, which then reproduce and pass on their genes.

For this reason, Bt crop technology sometimes enjoys short-term success in controlling pests but is soon undermined by the emergence of pests resistant to the toxin.[43,44,45] By 2009, the western corn rootworm had evolved resistance to a Bt maize specifically engineered to target the pest that was first commercialised only six years previously.[46] Bt-resistant rootworm populations have been reported in Iowa[46,47] and Illinois.[48]

5.4.2. The "refuge" concept breaks down

Farmers are encouraged to plant "refuges" of non-Bt crops as a resistance management strategy to delay the emergence of Bt-resistant pests. The idea is that the non-Bt crop acts as a refuge where Bt-susceptible pests can survive, ensuring the existence of a population of Bt-sensitive pests to mate with any Bt-resistant pests that survive in the adjacent field where the Bt crop is under cultivation. The theory is that the Bt-susceptible pest population will dilute out the Bt-resistant population that survives in the Bt crop, assuring that the predominant population is Bt-susceptible.

But a study on rootworm resistance in Iowa found that refuges were redundant in the case of substantial Bt-resistant rootworm populations, as the pests were able to live and reproduce in Bt maize fields. The study concluded, "Even with resistance

management plans in place, sole reliance on Bt crops for management of agriculture pests will likely hasten the evolution of resistance in some cases."[46]

Also, the effectiveness of refuges relies on the Bt crops expressing doses of Bt toxin that are high enough to kill pests, and the non-Bt refuges remaining free from Bt toxin-expressing genes. But cross-pollination between GM Bt maize has been found to cause "low to moderate" Bt toxin levels in the refuge plants,[49] making refuges less effective.

5.4.3. Secondary pests attack Bt crops

Nature abhors a vacuum. So even when Bt toxin succeeds in controlling a primary pest, secondary pests move into the ecological niche. For instance, in the United States, the Western bean cutworm has increased significantly in Bt maize fields.[50] In China and India, Bt cotton was initially effective in suppressing the target pest, the boll weevil. But secondary pests that are resistant to Bt toxin, especially mirids and mealy bugs, soon took its place.[51,52,53,54,55,56]

Two studies from China on GM Bt insecticidal cotton show that GM Bt technology is already failing under the onslaught of secondary pests:

A study of 1,000 farm households in five provinces found that farmers noticed a substantial increase in secondary pests after the introduction of Bt cotton. The researchers found that the initial reduction in pesticide use in Bt cotton cultivars was "significantly lower than that reported in research elsewhere" and that "more pesticide sprayings are needed over time to control emerging secondary pests" such as aphids, spider mites, and lygus bugs. In addition, a quarter of the farmers thought Bt cotton yielded less than non-GM varieties. Close to 60% said that overall production costs had not decreased, due to the higher price of Bt cotton seed.[57]

Field trials conducted over ten years in northern China show that mirid bugs have increased in cotton and multiple other crops, in proportion to a regional increase in Bt cotton adoption. The researchers' analyses show that "Bt cotton has become a source of mirid bugs and that their population increases are related to drops in [chemical] insecticide use in this crop." Moreover, mirid bug infestation of other food crops (Chinese dates, grapes, apples, peaches, and pears) increased in proportion to the regional planting area of Bt cotton.[58]

It is clear from these developments that GM Bt technology is not a "silver bullet" solution but is economically and environmentally unsustainable, as farmers who have paid premiums for Bt insecticidal seed have had to return to spraying costly and toxic pesticides.

5.4.4. Bt cotton farmers don't always give up insecticides

GM proponents often assume that farmers who adopt Bt crops give up chemical insecticides – but this is not necessarily the case. Tabashnik (2008) reported that while bollworms have evolved resistance to Bt toxin in one type of GM cotton, this has not caused widespread crop failure because "insecticides have been used from the outset" to control the pest.[45] So claims of reductions in insecticide use from Bt crop adoption are unreliable unless there is evidence that the farmer does not use chemical insecticides.

Moreover, most Bt crops currently commercialised or in the pipeline have added herbicide tolerance traits and so are likely to be grown with herbicides.[59] It is with good reason that one independent scientist

Pesticide use number-crunching

The most optimistic claim for reduced pesticide use from GM crops, in a paper by the private consultancy firm to the GM industry, P G Economics, and based on "farm-level impact data" from an unnamed source, is 6.9%.[41]

In 2008 in the US, according to official government data, GM crop acres required over 26% more pounds of pesticides per acre than acres planted to conventional varieties.[9]

A 2011 study by French government scientists found that pesticide use could be reduced by 30% without impairing yields or farm income[42] – and without GM crops.

has called GM crops "pesticide plants".[60]

5.4.5. Hidden chemical insecticides in Bt maize

Studies claiming reductions in insecticide use due to Bt crops have previously focused on insecticides that are applied to the soil or sprayed onto the plant after it has begun to grow. They may neglect to mention a different, potentially environmentally destructive type of pesticide: those that are applied to the seed before it sprouts.

According to a study by US entomologists, all commercially available rootworm-directed Bt maize seed is now treated before it is planted with the controversial chemical insecticides known as neonicotinoids. The authors suggested that the adoption of Bt maize "may shift insecticide use patterns" from sprayed insecticides to such seed treatments.[61]

So GM Bt crops may have done little more than help cause a shift in the type and means of application of chemical insecticide, rather than reducing or eliminating such chemicals. Where insecticides used to be applied to the soil or the plant while it is growing, now they are applied to the seed before planting.

Dr Doug Gurian-Sherman, senior scientist at the Union of Concerned Scientists, commented that neonicotinoid treatments on Bt maize seed aim to kill the insect pests that are not well controlled by Bt toxins. He added that these seed treatments are not confined to Bt maize: most maize seed, apart from organic, and an increasing proportion of the seed of other row crops, is now routinely treated with neonicotinoids.[62,63]

Neonicotinoids are systemic insecticides, meaning that they spread throughout all tissues of the crop plant as it grows and are even present in the pollen and nectar. Like the Bt toxin engineered into GM plants, neonicotinoids differ from sprayed insecticides in that they are persistently present in the growing plant and always active. Because of this long exposure period, pests are more likely to develop resistance to them, and non-target and beneficial insects are more likely to be exposed, too.

Neonicotinoids are toxic to a wide variety of beneficial creatures, including some that help protect crops.[64,65] They have been found to have highly toxic effects even at very low doses because they persist over long periods in soil and water.[66] The rise in the use of neonicotinoid seed treatments has been implicated in bee die-off and colony collapse.[67,68] Bees living near agricultural fields have been found to be exposed by multiple routes, including contaminated wild flowers growing near fields, and neonicotinoids have been found in dead bees.[68]

The chief – seemingly the only – concern of defenders of Bt crop technology is the volume of insecticide applied as sprays after planting. If that volume decreases, they consider that Bt crops reduce insecticide use. But they are not reporting the whole story. The case of neonicotinoid seed treatments shows that it is necessary to consider other types of insecticide applications, how toxic the insecticides are (based on peer-reviewed research, not industry data), how they behave and persist in the environment, and the acreage over which they are applied.[62]

Given the extreme toxicity of neonicotinoids to bees and other beneficial organisms, their high degree of persistence and spread, and the vast acreage over which they are applied, it is questionable whether seed-treated Bt crops have had a beneficial effect on insecticide use.

5.4.6. Conclusion

Studies claiming that Bt crops reduce insecticide use have failed to take into account important aspects such as:

- The toxicity to non-target and beneficial organisms of the engineered Bt toxins
- The amount, type, and toxicity of insecticides actually used by farmers in the field even when Bt seeds are used – reflecting pest resistance and ineffectiveness of refuges
- Changes in the way insecticides are used, such as the transition from sprayed pesticides to use of insecticidal seed treatments.

Also, when evaluating the impact of GM Bt crops on insecticide use, a more useful comparator than chemically-grown non-GM crops would be non-GM crops under organic or integrated pest management, where insecticide use is reduced or eliminated. This would quickly make clear which farming methods can best reduce insecticide use while maximizing yield and farmer incomes.

5.5 **Myth:** GM Bt crops only affect target pests and their relatives **Truth:** GM Bt crops are not specific to pests but affect a range of organisms

GM proponents claim that Bt crops only affect target pests and their close relatives. Regulators have uncritically accepted this claim and allowed the commercialisation of Bt crops with a minimum of oversight. But research studies show that this assumption is false.

5.5.1. Bt crops harm soil organisms

Mycorrhizal fungi benefit plants by colonising their roots, helping them take up nutrients, resist disease, and tolerate drought. A study comparing Bt and non-Bt maize found a lower level of mycorrhizal colonisation in the roots of Bt maize plants. Residues of Bt maize plants, ploughed under at harvest and kept mixed with soil for up to four months, suppressed soil respiration (carbon dioxide production), markedly altered bacterial communities, and reduced mycorrhizal colonisation.[69] A separate field study on Bt maize residues ploughed into soil after harvest confirmed that Bt toxin resisted breakdown and persisted in soil for months.[70]

Arbuscular mycorrhizal fungi (AMF) are beneficial fungi that penetrate the root cells of the host plant. Bt maize has been found to decrease arbuscular mycorrhizal fungi (AMF) colonisation of the roots, compared with non-GM maize.[71,72]

5.5.2. Bt crops harm non-target and beneficial insects

GM Bt insecticide-producing crops have been found to have toxic effects on non-target insect populations,[73] including butterflies[74,75,76] and beneficial pest predators such as ladybirds[77,78] and lacewings.[79] Bt crops have more negative than positive impacts on beneficial insects.[80] Bt toxin impacts bee learning behaviour, interfering with bees' ability to find nectar sources for food.[81]

5.5.3. Bt crops harm aquatic organisms

A study conducted in Indiana, USA found that Bt insecticide released from GM Bt maize was polluting 25% of streams tested.[82] Other studies have found that GM Bt maize biomass is toxic to aquatic[83] and soil organisms.[69] Water fleas (an organism often used as an indicator of environmental toxicity) fed GM Bt maize showed toxic effects including reduced fitness, higher mortality, and impaired reproduction.[84]

5.5.4. Conclusion

Bt crops are not specific to the target pests and close relatives but negatively affect a range of non-target organisms, including beneficial insects that help protect crops.

5.6 **Myth:** Roundup is a benign and biodegradable herbicide **Truth:** Roundup persists in the environment and has toxic effects on wildlife

Manufacturers claim that Roundup, the glyphosate-based herbicide used on most GM crops, breaks down quickly and harmlessly in the environment. But research shows that this is untrue:

- In soil, glyphosate has a half-life (the length of time taken to lose half its biological activity) of between 3 and 215 days, depending on soil conditions.[85,86] In water, glyphosate's half-life is 35–63 days.[87]
- Although glyphosate binds well to soil particles, the Danish National Pesticide Monitoring Program showed that glyphosate and its main breakdown product AMPA are washed out of the root zone of clay soils in concentrations that exceed the acceptable quantities for drinking water (0.1 µg/l), with maximum values of over 5 µg/l.[88]
- Glyphosate was detected in between 60 and 100% of air and rain samples taken in the American Midwest during the crop growing season in the American Midwest, where Roundup Ready GM crops are widely planted.[89]
- Glyphosate and its main breakdown product, AMPA, were detected in streams in the American Midwest during the crop growing season.[90]
- Glyphosate is toxic to earthworms[91] and reduces bird populations due to habitat changes.[92]
- Roundup is highly toxic to amphibians. A study in a natural setting found that Roundup application at the rate recommended by the manufacturer eliminated two species of tadpoles and nearly exterminated a third species, resulting in a 70% decline in the species richness of tadpoles. Contrary to common belief, the presence of soil does not reduce the chemical's effects.[93] Further experiments with lower concentrations, well within levels to be expected in the environment, still caused 40% amphibian mortality.[94]
- Claims that Roundup and glyphosate are safe for human health and the environment have been overturned in courts in the United States[95] and France. The French court forced Monsanto to withdraw advertising claims that Roundup is biodegradable and leaves the soil clean after use.[96]

Regulatory bodies around the world have not caught up with the state of the science on Roundup and glyphosate. Instead they continue to rely on decades-old studies, mostly sponsored by manufacturers, to claim it is safe. An objective up-to-date review of Roundup and glyphosate's persistence and toxicity is long overdue.

5.7 **Myth:** Roundup is a benign herbicide that makes life easier for farmers
Truth: Roundup causes soil and plant problems that impact yield

GM Roundup Ready crops are marketed on the basis that Roundup is a safe herbicide that simplifies weed control and makes the farmer's life easier. But recent studies show that Roundup and glyphosate can accumulate in plants, have negative effects on soil organisms, and harm the growth and health even of soy plants that are genetically engineered to tolerate it. These effects may be partly responsible for yield decline and disease outbreaks found in GM Roundup Ready soy and maize.

5.7.1. Glyphosate causes or exacerbates plant diseases

> *"When you spray glyphosate on a plant, it's like giving it AIDS."*
> – Michael McNeill, agronomist and farm consultant[97]

Manufacturers claim that glyphosate kills plants by inhibiting an enzyme necessary for plant growth. But research shows that glyphosate has another way of killing plants: it makes the plant more susceptible to disease, potentially leading to the plant's death from the disease. Spraying glyphosate on a plant is, as US agronomist Michael McNeill said, "like giving it AIDS".

One possible mechanism for this process is offered in a study on GM RR soybeans. The study found that once glyphosate is applied to the plant, it accumulates in the plant tissues and then is released into soil through the roots. There, it stimulates the growth of certain fungi, notably Fusarium, a fungus that causes wilt disease and sudden death syndrome in soy plants.[98] Other studies confirm the link between glyphosate applications and increased infection with Fusarium.[99,100,101,102,103]

Interestingly, one study found that Fusarium colonisation of roots was greater in GM RR soy compared with non-GM soy even when glyphosate is not applied. The researchers suggested that this was due to an unintended change in the GM crop brought about by the genetic engineering process.[98]

Fusarium is of especial concern because it does not only affect plants. It produces toxins that can enter the food chain and harm humans[104] and livestock. In pigs, Fusarium-contaminated feed impairs reproduction[105] and increases stillbirths.[106]

Glyphosate has also been shown to increase the incidence and severity of other fungal diseases in plants, including take-all in wheat and Corynespora root rot in soy.[107,108]

In an attempt to combat soil-borne diseases such as Fusarium, Monsanto markets its new Roundup Ready 2 Yield soy seed with a proprietary fungicide/insecticide coating.[109] In other words, Monsanto has created a problem (fungal infection) by genetically modifying the soy seeds and is then profiting from a techno-fix "solution" to that problem. Such chemical treadmills are profitable for seed and chemical companies, but hurt farmers, consumers, and the environment.

5.7.2 Glyphosate makes nutrients unavailable to plants

Glyphosate binds vital nutrients such as iron, manganese, zinc, and boron in the soil, preventing plants from taking them up.[110] So GM soy plants treated with glyphosate have lower levels of essential nutrients and reduced growth, compared with GM and non-GM soy controls not treated with glyphosate.[111] Lower nutrient uptake may partly account for the increased susceptibility of GM soy to disease, as well as its lower yield. It could also have implications for humans and animals that eat the crop, as it is less nutritious.

5.7.3 Glyphosate impairs nitrogen fixation

The yield decline in GM RR soy may be partly due to glyphosate's negative impact on nitrogen fixation, a process that is vital to plant growth and depends on the beneficial relationship between the soy plants and nitrogen-fixing bacteria. In young RR soy plants, glyphosate has been found to delay nitrogen

fixation and reduce the growth of roots and sprouts, resulting in yield decline. In drought conditions, yield can be reduced by up to 25%.[112]

The mechanism may be explained by another study, which found that glyphosate enters root nodules and negatively affects beneficial soil bacteria that are essential for the nitrogen fixation process. It inhibits root development, reducing root nodule biomass by up to 28%. It also reduces by up to 10% an oxygen-carrying protein, leghaemoglobin, which helps bind nitrogen in soybean roots.[113]

To counter such problems, seed and agrochemical companies have begun to market a "techno-fix" in the form of nitrogen-fixing bacterial inoculants, which are either applied to soy seed before sale or to the soil after sowing. The companies claim that this will increase yield potential.[114] However, a soybean inoculant evaluation trial conducted in Iowa concluded, "none of the inoculants resulted in a significant yield increase over the non-inoculated plots".[115] Inevitably, the cost of such treatments, even when they do not work, are borne by farmers.

5.7.4. Conclusion

Roundup and other glyphosate herbicides are not benign but have negative effects on soil and crops, some of which impact plant health and yield. Glyphosate's link with Fusarium infection is especially serious as Fusarium can harm humans and livestock.

5.8 **Myth:** GM crops help biodiversity
Truth: The herbicides used with GM crops harm biodiversity

> *"Many farmland birds rely on seeds from weeds for their survival and the [UK] government's farm scale trials showed that GM beet and GM spring oilseed rape [canola] reduced seed numbers by up to 80% compared with conventional beet and oilseed rape. The commercialisation of GM beet and oilseed rape could be disastrous for birds. The government is committed to reversing bird declines and has promised to ban GM crops if they damage the environment. The Farm Scale Evaluations (FSEs) show that two GM crops harm the environment and ministers now have no choice but to refuse their approval."*
>
> – Dr Mark Avery, director of conservation at the UK's Royal Society for the Protection of Birds (RSPB) and member of the UK government's Science Review Panel[116]

In the early 2000s the UK government conducted three-year farm-scale trials to examine the impacts of managing GM herbicide-tolerant crops (maize, sugar beet and canola) on farmland biodiversity. Each field was divided in half, with one half planted with a non-GM variety managed according to the farmer's normal practice, and the other half planted with a GM herbicide-tolerant variety. The GM beet was tolerant to the glyphosate-based herbicide Roundup and the GM maize and canola were tolerant to glufosinate ammonium. The herbicide-tolerance genes enabled farmers to spray the crops with these broad-spectrum (kill-all) herbicides, killing all weeds but allowing the crop to survive.

Weeds provide food and habitat for birds, insects, and other wildlife, so the farm-scale trials recorded levels of weeds and invertebrates in the fields and field margins. Selected groups of other organisms with wider foraging ranges (beetles, bees, and butterflies) were also studied. The trials looked at whether the changes in management associated with GM crops would reduce weed levels and have wider impacts on farmland biodiversity.

The findings showed that the cultivation of GM herbicide-resistant crops reduces wildlife populations and damages biodiversity, due to the effects of the broad-spectrum herbicides with which they are grown.[117,118,119,120,121,122]

GM herbicide-resistant maize was found to be better for wildlife than non-GM maize, with more weed species and insects in and around the field.[117,118,119,120,121,122] But the GM maize was measured against a non-GM maize grown with atrazine, a toxic herbicide that was banned in Europe soon after the trials ended. With such a toxic control, it was highly likely that the GM maize would be found to be better for wildlife.A more useful comparator would have been a maize grown in an organic or integrated pest management (IPM) system, which eliminate or reduce herbicide use.

In the EU, this is not a purely idealistic notion. A 2009 European Directive asks member states to implement national plans to adopt integrated pest management and alternative approaches in order to reduce pesticide use.[123]

5.9 **Myth:** GM crops bring economic benefits to farmers **Truth:** Economic impacts of GM crops on farmers are variable

> *"Perhaps the biggest issue raised by these results is how to explain the rapid adoption of GE crops when farm financial impacts appear to be mixed or even negative."*
>
> – J. Fernandez-Cornejo, W. D. McBride, The adoption of bioengineered crops, US Department of Agriculture[6]

The question of economic impacts of GM crops on farmers is complex and a thorough examination is beyond the scope of this report. Results vary and depend on many factors, including:

- Suitability of the crop for local conditions
- Climate
- Pest and disease prevalence
- Cost of weed management
- Subsidies and incentives offered by governments or corporations
- Cost of seed
- Availability of markets for the crop.

The following studies give an overview of the issue.

Fernandez-Cornejo (2002)

This report on farm-level economic impacts of adopting GM crops found that they were "mixed or even negative". The report, mostly based on data from USDA surveys, found that adoption of herbicide-tolerant maize had a positive effect on net returns, but the effect was negative for Bt maize. GM soybeans had no effect either way.[6]

Gómez-Barbero (2006)

This review for the European Commission of the economic impact of the main GM crops worldwide found that herbicide-tolerant soybeans had a negative effect on US farmers' income. But the same crop brought income gains to Argentine farmers, due to lower prices for GM seed in that country.[124]

Why do US farmers adopt GM soy if it brings no financial gain? The authors suggested that the reason may be simpler weed control,[124] though the data cited to back up this claim pre-date the explosion of herbicide-resistant superweeds that has caused the cost of GM soy production to rise (see 5.2).

The review found that Bt cotton in China had produced economic gains for farmers, mostly because of reduced expenditure on pesticide sprays. Bt cotton in India was claimed to provide economic benefits, though with considerable "local variability".[124] These studies were also carried out before the full impact of pest resistance and emergence of secondary pests was experienced by Chinese and Indian farmers.

Morse (2005)

This study found that Bt cotton in India produced better profit margins for farmers than non-GM cotton. However, the authors pointed out that these benefits will only be sustained if pests do not evolve resistance to Bt cotton.[125] Recent studies suggest that they are already evolving resistance (see 5.4).

These findings are confirmed by a leaked advisory from the Indian government which blamed Bt cotton for the spate of farmer suicides across the subcontinent. The advisory stated, "Cotton farmers are in a deep crisis since shifting to Bt cotton. The spate of farmer suicides in 2011–12 has been particularly severe among Bt cotton farmers." The advisory added that Bt cotton's success had only lasted five years. Since then, yields had fallen and pest attacks had increased: "In fact cost of cotton cultivation has jumped... due to rising costs of pesticides. Total Bt cotton production in the last five years has reduced."[126]

5.9.1. The rising cost of GM seed

An important factor in assessing the economic impact of GM crops is the cost of seed. In the United States, where GM firms dominate the seed market, a 2009 report documents that prices for GM seeds have increased dramatically compared with prices for non-GM and organic seeds. This cut average farm incomes for US farmers growing GM crops. The $70 per bag price set for RR2 soybeans

for 2010 was twice the cost of conventional seed and reflected a 143% increase in the price of GM seed since 2001.[127]

US farmers have grown increasingly concerned about the high price and poor performance of GM seed. A 2011 media report said that the seed companies had responded by withdrawing a high-performing non-GM variety of maize, which gave higher yields than GM varieties. The report added that the companies are hiking the prices of herbicides used by non-GM farmers to artificially increase the cost of non-GM production.[128]

Farmers have little choice but to tolerate such price hikes because of consolidation within the seed industry. In other words, the GM industry dictates which seed varieties are available. In 2008, 85% of GM maize patents and 70% of non-maize GM plant patents in the US were owned by the top three seed companies: Monsanto, DuPont, and Syngenta. Even these three companies are not independent of each other but increasingly network to cross-license GM seed traits.[131]

The largest of the big three companies is Monsanto. In 2010 Monsanto raised its prices for its RR2 soybeans and SmartStax maize seeds so steeply that the US Department of Justice launched an investigation into the consolidation of agribusiness firms that has led to anti-competitive pricing and monopolistic practices. Farmers actively gave evidence against companies like Monsanto.[132,133]

The same pattern has been reported in India. Moreover, as prices of GM Bt cotton seed have escalated,[134] non-GM varieties – in some cases better-performing than the GM varieties – have been withdrawn from the market.[135,136] The result is that farmers are forced into dependency on the GM industry. Such reports expose claims that GM crops increase "farmer choice" as misleading.

5.9.2. Conclusion

The economic impacts of GM crops on farmers are variable and depend on complex factors. However, consolidation in the seed market has led to steep increases in the price of GM seed as compared with non-GM seed. This consolidation has also led to competing high-performing non-GM seed varieties being withdrawn from the market, restricting farmer choice.

The importance of independent information

Some who claim that GM crops bring economic benefits to farmers cite upbeat reports written by Graham Brookes and Peter Barfoot. But such reports are not independent. Brookes and Barfoot are the directors of a private consultancy firm called PG Economics, which has GM and agrochemical firms as its primary clients.[129] Generally, PG Economics' reports are commissioned by GM firms or industry lobby groups such as Agricultural Biotechnology in Europe,[130] whose members include the large GM seed companies. Most PG Economics reports are not peer reviewed and rely heavily on industry data.

5.10 **Myth:** GM crops can "coexist" with non-GM and organic crops **Truth:** Co-existence means widespread contamination of non-GM and organic crops

> *"OK, we know that cross-pollination will occur but we've got thirty years of experience to say we know how far pollen will travel. And therefore what we've done is we'll grow a GM crop at a distance away from a non-GM crop, so the people that want non-GM can buy non-GM, and the people that want GM can buy GM. The two will not get mixed up. Everybody will have the right to choose."*
>
> – Paul Rylott, seed manager for Aventis CropScience (now Bayer)[137]

The GM industry used to claim that GM contamination of non-GM crops could not occur. After it became clear that this was false, it shifted the argument to lobbying for "co-existence" of GM, non-GM, and organic crops. The industry now argues that farmers should be able to choose to plant GM crops if they wish and says that no serious problems are caused for non-GM and organic farmers.

But experience has shown that the arrival of GM crops in a country removes choice. "Coexistence" rapidly results in widespread contamination of non-GM crops, resulting in lost markets. Contamination occurs through cross-pollination, spread of GM seed by farm machinery, and inadvertent mixing during storage. Farmers are gradually forced to grow GM crops or have their non-GM crops contaminated.

Scientific studies confirm that GM contamination is unavoidable once GM crops are grown in a region. For example, GM herbicide-tolerant oilseed rape (canola) seed can persist and remain viable in soil for years. GM herbicide-resistant "volunteers" – plants that were not deliberately planted but are the result of germination of residual GM seeds from crops previously grown in the field – were found growing ten years after the GM oilseed rape crop had been planted.[138] GM herbicide-resistant oilseed rape was found to be thriving in the wild in North Dakota, often far from areas of agricultural production. GM genes were present in 80% of the wild canola plants found.[139,140]

5.10.1. Who is liable for GM contamination?

In countries where legal liability for GM contamination is clearly established, GM crop cultivation has become severely restricted. In Germany, a law has been passed making farmers who grow GM crops liable for economic damages to non-GM and organic farmers resulting from GM contamination.[141,142] The law has virtually halted the planting of GM crops in the country because farmers are not prepared to accept liability for contamination.[142]

The fact that farmers who previously chose to grow GM crops have ceased to do so because of the fact that they could be held liable for damages is clear evidence that coexistence is impossible. In light of this, it is not surprising that the GM seed industry has lobbied forcefully against the implementation of similar liability laws in the US and Canada.

The GM seed industry also knows it cannot contain or control its GM genes. In February 2011, after years of industry lobbying, the EU dropped its policy of zero tolerance of animal feed with unapproved GMOs, allowing contamination of up to 0.1%.[143,144] In doing so, it granted industry release from liability for damages resulting from GM contamination with up to 0.1% of GM crop varieties ("Low Level Presence") that are under evaluation but not yet approved in the EU.

In the United States, federal courts have recognised that GM crops are likely to contaminate non-GM crops. Two court rulings reversed US Department of Agriculture (USDA) approvals for the commercial planting of GM sugar beet and GM alfalfa. The courts ordered the USDA to halt planting of the GM crops until it had completed an environmental impact statement

(EIS) on the environmental and economic effects of contamination of non-GM crops.

In the case of GM sugar beet, the USDA defied the court order and allowed farmers to continue planting the crop while it worked on the EIS. In the case of GM alfalfa, USDA completed an EIS in which it admitted that cross-contamination with non-GM alfalfa could occur and that the economic interests of non-GM growers could be harmed. But, bowing to heavy lobbying from the GM industry, USDA "deregulated" GM alfalfa, an action that superseded the court ruling and allowed planting of the crop without restriction.[145]

5.11 **Myth:** If GM contamination occurs, it is not a problem **Truth:** GM contamination has had severe economic consequences for farmers, food and feed companies, and markets

> *"If some people are allowed to choose to grow, sell and consume GM foods, soon nobody will be able to choose food, or a biosphere, free of GM. It's a one way choice, like the introduction of rabbits or cane toads to Australia; once it's made, it can't be reversed."*
>
> – Roger Levett, specialist in sustainable development[163]

GM contamination of crops has had severe economic consequences, threatening the livelihoods of farmers who receive premiums for growing organic and GM-free crops and blocking export markets to countries with strict regulations on GMOs.

Examples of GM contamination problems include:

- In 2011 an unauthorized GM Bt pesticidal rice, Bt63, was found in baby formula and rice noodles on sale in China.[146] Contaminated rice products were also found in Germany[147] and Sweden.[148] The same rice was found in rice products in New Zealand in 2008, leading to product recalls.[149] GM Bt rice has not been shown to be safe for human consumption. Periodic recalls of products contaminated with Bt63 rice continue to be reported even today in Europe.
- In 2009 an unauthorized GM flax called CDC Triffid contaminated Canadian flax seed supplies, resulting in the collapse of Canada's flax export market to Europe.[150,151]
- In 2006 an unapproved experimental GM rice, grown only for one year in experimental plots, was found to have contaminated the US rice supply and seed stocks.[152] Contaminated rice was found as far away as Africa, Europe, and Central America. In 2007 US rice exports were down 20% from the previous year as a result of the GM contamination.[153] In 2011 the company that developed the GM rice, Bayer, agreed to pay $750 million to settle lawsuits brought by 11,000 US farmers whose rice crops were contaminated.[154] A court ordered Bayer to pay $137 million in damages to Riceland, a rice export company, for loss of sales to the EU.[155]
- In Canada, contamination from GM oilseed rape has made it virtually impossible to cultivate organic, non-GM oilseed rape.[156]
- Organic maize production in Spain has dropped as the acreage of GM maize production has increased, due to contamination by cross-pollination with GM maize.[157]
- In 2000 GM StarLink maize, produced by Aventis (now Bayer CropScience), was found to have contaminated the US maize supply. StarLink had been approved for animal feed but not for human consumption. The discovery led to recalls of StarLink-contaminated food products across the US, spreading to Europe, Japan, Canada, and other countries. Costs to the food industry are estimated to have been around $1 billion.[158] In addition, the US government bore indirect costs of between $172 and $776 million through the USDA's Loan Deficiency Payments Program, which offers producers short-term loans and direct payments if the price of a commodity crop falls below the loan rate.[159] Aventis paid out $110 million to farmers who brought a class action suit against the company[160] and spent another $110 million buying back StarLink-contaminated maize.[152]

As no official body keeps records of GM contamination incidents, Greenpeace and Genewatch UK have stepped into the gap with their GM Contamination Register.[161] In the years 2005–2007 alone, 216 contamination incidents were recorded in the database.[162]

5.12 **Myth:** Horizontal gene transfer from GM crops is unlikely or of no consequence

Truth: GM genes can escape into the environment by horizontal gene transfer with potentially serious consequences

Most GM contamination incidents occur through cross-pollination, contamination of seed stocks, or failure to segregate GM from non-GM crops after harvest. But for years, scientists have warned that GM genes could also escape from GM crops into other organisms through a mechanism called horizontal gene transfer (HGT). HGT is the movement of genetic material between unrelated species through a mechanism other than reproduction. Reproduction, in contrast, is known as vertical gene transfer because the genes are passed down through the generations from parent to offspring.

GM proponents and government regulators often claim that, based on available experimental data, HGT is rare. The EU-supported website GMO Compass states, "So far, horizontal gene transfer can only be demonstrated under optimised laboratory conditions."[164] Alternatively, they argue that if it does happen, it does not matter, as GM DNA is no more dangerous than non-GM DNA.

But there are several mechanisms through which HGT can occur, some of which are more likely than others. HGT via some of these mechanisms occurs easily and frequently in nature. The consequences of HGT from GM crops are potentially serious, yet have not been adequately taken into account by regulators.

The basic mechanisms by which HGT could occur are:

- Uptake of GM DNA by bacteria
- Uptake of DNA from the digestive tract into the tissues of the organism
- Transmission of GM DNA via Agrobacterium tumefaciens, a bacterium that is often used to introduce GM genes into plants because of its natural ability to carry and transfer foreign DNA and to infect plants through wounds in their outer layer
- Gene transfer by viruses.

The following sections outline these mechanisms and provide a perspective on the frequency at which these events can occur, as well as their potential impacts.

5.12.1. DNA uptake by bacteria

Bacteria are promiscuous. They are always exchanging DNA between themselves and taking up DNA from their environment. Some of this environmentally acquired DNA can be incorporated to their genome and may be expressed. There are two scenarios in which DNA uptake by bacteria could result in HGT of GM genes.

The first is the transfer of GM DNA from GM food into intestinal bacteria. DNA from a GM plant is released into the intestinal tract of the consumer during digestion. Contrary to frequent claims, GM DNA is not always broken down in digestion and can survive in sufficiently large fragments that can contain intact genes that are potentially biologically active (see 3.1.1, 3.6.2).

Bacteria of many different species are present in the digestive tract, some of which can take up DNA from their environment and incorporate it into their own DNA. In the case of GMOs, this could be problematic. For example, if the GM plant contained a gene for antibiotic resistance, the bacterium could incorporate that antibiotic resistance gene into its genome, and thereby become resistant to the antibiotic. If the bacteria in question happened to be pathogenic (disease-causing), this process would have created an antibiotic-resistant pathogen – a "superbug".

Since bacteria in the intestinal tract frequently exchange DNA, the creation of a superbug could be a two-stage process. First, the antibiotic resistance gene could initially be taken up and incorporated into a non-pathogenic bacterium in the intestinal tract. Subsequently, if a pathogenic bacterial species becomes part of the intestinal flora, the non-pathogenic bacterium could transfer the antibiotic resistance gene to the pathogenic

bacterium, thereby creating a "superbug".

The transfer of GM genes from food to intestinal bacteria has been documented in a study on humans, which found that the intestinal bacteria of a person whose diet included soy carried sequences unique to the GM soy that was part of their diet.[165]

The second scenario in which DNA uptake by bacteria could result in HGT of GM genes is the transfer of GM DNA to soil bacteria. Cultivation of transgenic crops leads to the degradation of GM plant material in the environment, liberating GM genes into the soil. Every cubic centimetre of soil contains thousands of different species of bacteria, only a small percentage of which have been identified and characterised. Some of the known soil bacteria can, and do, take up free DNA that may be present in the soil, incorporating that DNA into their genomes.[166] This could result in the transfer of GM genes to natural soil bacterial populations. Based on limited currently available data, this type of event has been calculated to be extremely rare.[167] However, it has been shown that GM DNA can persist in soil at detectable levels for at least a year,[168] increasing the likelihood of HGT.

In addition, we only know a small fraction of the soil bacteria that could potentially take up DNA from their environment.[166] Furthermore, if the uptake of a GM gene, for example for antibiotic resistance, gives the bacterium a survival and growth advantage, this can allow them to outcompete other bacterial strains in the presence of widely used antibiotics in agriculture and medicine. Therefore, this initial rare event could still result in a significant environmental and health outcome.[169]

5.12.2. DNA uptake during digestion of GM foods

A study on mice demonstrated that foreign DNA present in food can be transferred from the digestive tract to the bloodstream of animals that eat the food. This foreign DNA was also found in white blood cells and in the cells of many other tissues of the mice.[170] In a separate study, foreign DNA in a diet fed to pregnant mice was found in the organs of their foetuses and newborn offspring. The foreign DNA was believed to have reached the foetus through the placenta.[171]

It has also been shown that GM DNA in feed can be taken up in the organs of the animals that eat it and can be detected in the meat and fish that people eat.[172,173,174,175]

Most of the GM DNA in food is fragmented before it reaches the blood or tissues. However, a few copies of GM DNA large enough to contain the sequence of a full and functional gene will also be present in the digestive tract and can be taken up into the blood at lower frequency, where it can be transported by the blood and taken up by cells of some tissues or organs.[170] Once taken up by a cell, such a GM gene could be integrated into the DNA of the cell, causing either direct mutation of a host gene function or reprogramming the host cell to produce the protein for which that GM gene codes, or both.

At present, this scenario is speculative. Although it is clearly possible to detect transgenic DNA in the tissues of organisms that consume GM feed, no research has been published that shows that the GM DNA is expressed in the tissues of those organisms. It would be expected that if such expression did occur, it would not occur frequently. In order to find out whether such expression events actually occur, it would be necessary to conduct very large-scale studies – though identifying a suitable experimental design would be challenging.

It should be pointed out, however, that although such events may be of low frequency, because of the widespread consumption of GMOs by both humans and animals, the fact that such events are of low frequency does not eliminate them as important to the biosafety assessment of GMOs.

Though the mechanism is still unclear, GM feed has been found to affect the health of animals that eat it. GM DNA from soy was detected in the blood, organs, and milk of goats. An enzyme, lactic dehydrogenase, was found at significantly raised levels in the heart, muscle, and kidneys of young goats fed GM soy.[176] This enzyme leaks from damaged cells during immune reactions or injury, so high levels may indicate such problems.

5.12.3. Horizontal gene transfer by Agrobacterium tumefaciens

Agrobacterium tumefaciens (A. tumefaciens) is a soil bacterium that is often used to introduce GM genes into plants.

The introduction of GM genes into plants by infection with A. tumefaciens is carried out by exploiting a Ti plasmid – a small circular molecule of DNA that is naturally found in A. tumefaciens. When A. tumefaciens infects a plant, the Ti plasmid is introduced into the plant cells. Parts of the Ti plasmid may then insert themselves into the DNA of the plant.

Plant biotechnologists have adapted this natural process in order to introduce foreign DNA into plants and thereby produce GM crops. First, the naturally occurring genes of the Ti plasmid in the region that can insert into host plant cell DNA are removed and replaced with the GM gene of choice. The now genetically modified Ti plasmid is then introduced into A. tumefaciens, which in turn is used to infect plant cells. Once inside the plant cell, some of the genetically modified Ti plasmid can insert into host plant cell DNA, thereby permanently altering the genetic makeup of the infected cells.

Although A. tumefaciens is a convenient way of introducing new genes into plants, it can also serve as a vehicle for HGT from the GM plant to other species. This can happen via two mechanisms.

First, residual A. tumefaciens carried in a GM plant could infect plants of other species, thereby carrying the GM gene(s) from the intentionally genetically modified plant into other plants. A. tumefaciens can serve as a vehicle for HGT to hundreds of species of plants, since A. tumefaciens has been found to infect a wide range of plant species.

The second mechanism creates the risk that A. tumefaciens could pass GM genes on to an even wider range of species, including, but not limited to, plants. It consists of certain types of fungi functioning as intermediate hosts in the transfer of transgenes from GM A. tumefaciens to other organisms.

A 2010 study found that under conditions found in nature, A. tumefaciens introduced DNA into a species of disease-causing fungi that is known to infect plants. The study also found that GM DNA sequences in the A. tumefaciens were incorporated into the DNA of the fungi. In other words, the A. tumefaciens was genetically engineering the fungi.

The authors concluded that in cases where a GM plant is infected with fungi, A. tumefaciens in the GM plant could infect the fungi, introducing GM genes into the fungi.[177] Such fungi could, in turn, pass the GM genes onto other plants that they infect.

Genetic engineers had previously assumed that A. tumefaciens only infects plants. But this study showed that it can infect fungi, a different class of organism. The study stated, "A. tumefaciens may be able to [genetically] transform non-plant organisms such as fungi in nature, the implications of which are unknown."[177] The authors pointed out that A. tumefaciens is already known to transform – genetically modify – human cells in the laboratory.[177,178]

One of the study's co-authors, Andy Bailey, a plant pathologist at the University of Bristol, UK, said, "Our work raises the question of whether [A. tumefaciens's] host range is wider than we had thought – maybe it's not confined only to plants after all."[179]

The implications of this research are that it is possible that GM gene(s), once introduced by A. tumefaciens into a GM crop and released into the environment, could then be introduced into an organism outside the plant kingdom – in this case, a fungus – and genetically modify it. This would be an uncontrolled and uncontrollable process, with unpredictable consequences.

Implications of horizontal gene transfer through A. tumefaciens

Could A. tumefaciens transfer GM genes from a GM plant to another organism under realistic farming conditions? The answer depends on whether any A. tumefaciens carrying GM genes remains in the GM crop that is planted in open fields. Genetic engineers use antibiotics to try to remove the A. tumefaciens from the GM plant after the initial GM transformation process is complete in the laboratory. But this process has

been found to be unreliable and incomplete:

- A study on GM brassicas, potato and blackberry found that the use of three antibiotics failed to completely remove A. tumefaciens. Instead, the A. tumefaciens contamination levels increased from 12 to 16 weeks after the GM transformation process and the A. tumefaciens was still detected 6 months after transformation.[180]
- A study on GM conifers found that residual A. tumefaciens remained in the trees 12 months after the genetic transformation but were not detected after this time in the same plants.[181]

However, these experiments only examined the first GM plant clones. In the GM development process, such GM clones go through a long process of back-crossing and propagation with the best-performing non-GM or GM plant relatives in order to try to produce a GM plant that performs well in the field and expresses the desired traits. The important question is whether A. tumefaciens carrying GM genes survives this back-crossing and propagation process and remains in the final GM plant that is commercialised.

To the best of our knowledge there have been no studies to assess whether any A. tumefaciens remains in the final commercialised GM plant. The study on GM conifers examined the initial GM clones that were grown on, not plants that had been cross-bred and propagated over several generations, as GM crops are before they are commercialised, so it does not provide an answer to this question.

However, this question should be answered before a GM variety is commercialised, in order to avoid unwanted consequences that could be caused by residual A. tumefaciens in the final GM plant. Examples of consequences that should be excluded are the transfer of insecticidal properties to bacteria, or of herbicide tolerance to other crops or wild plants. The study discussed above (5.12.3) shows that the introduction of GM genes into crop plants could have consequences to organisms outside the plant kingdom, through the mechanism of infection by fungi carrying A. tumefaciens, which in turn carry GM genes.[177]

The consequences of such HGT for human and animal health and the environment are not predictable, but are potentially serious. The health and environmental risk assessment for any GM variety must demonstrate that the GM plants have been completely cleared of GM A. tumefaciens before they are approved for commercialisation.

5.12.4. Gene transfer by viruses

Viruses are efficient at transferring genes from one organism to another and in effect are able to carry out HGT. Scientists have made use of this capacity to create viral gene transfer vectors that are frequently used in research to introduce GM genes into other organisms. Such vectors based on plant viruses have also been developed to generate GM crops, though no crops produced with this approach have been commercialised to date.[182 183]

The viral vectors that are used to generate GM crops are designed to prevent the uncontrolled transfer of genetic material. However, because the long time period during which virally engineered crops would be propagated in the environment, and the large number of humans and livestock that would be exposed to this GM genetic material, there is a real, though small, risk that unintended modifications could occur that could lead to virus-mediated HGT – with unpredictable effects.

Another potential risk of virus-mediated HGT comes from GM crops engineered to contain a virus gene, in particular those carrying information for a viral "coat" protein. This is done in an attempt to confer resistance of the crop from actual infection and damage by the family of 'wild' virus from which the viral GM gene was derived. However, it has been suggested that if a GM crop containing a viral gene of this type was infected by the viruses, it may result in exchange of genetic material between the GM viral gene in the plant and the infecting virus, through a process known as recombination. This can potentially result in a new more potent ("virulent") strain of virus.[184,185]

The reasons for these concerns are as follows. The GM viral gene will be present in every single cell of the crop. As a result, the large-scale cultivation of such a viral GM gene-containing crop will result in an extremely high concentration of particular viral genes in fields. It has been suggested that this provides an unprecedented opportunity for genetic material recombination

events to take place between an infecting virus and GM viral genes in the crop, thereby increasing the risk of new, mutated, and potentially more virulent strains of virus being produced.[185]

Such viral mutation with increased virulence has been shown to occur under laboratory conditions.[186,187]

To date only two GM crops engineered with genes from viruses have been commercialised: a variety of squash grown in the USA and Mexico,[188] [189] and papaya cultivated in Hawaii.[190] There are no reports of any investigations to see if any new viral strains have arisen by recombination in these two crops. Interestingly, and quite unexpectedly, although the GM squash was resistant to viral infection, it was found to be prone to bacterial wilt disease following attack by beetles.[191]

5.12.5. Overall assessment of the risks of HGT by the above methods

HGT events of all types are of very low probability of occurrence. The method with the highest probability of occurring is DNA uptake by bacteria in either the environment or the digestive tract. There is good evidence that this has already happened in the intestinal bacteria of humans who consume GM soy.

The other scenarios are of significantly lower probability. However, given the extremely wide distribution of GM crops and their intended use over decades, these low probabilities translate into the likelihood that HGT events could actually occur even via the mechanisms that are expected to take place at lower probabilities.

Therefore, the negative impacts and risks associated with HGT must be taken into account in considering the overall biosafety of any GM crop.

Conclusion to Section 5

Most of the benefits for farmers and the environment claimed for GM crops are either exaggerated or false. For example, contrary to frequent claims, GM crops have not increased intrinsic yield. Crop yields have increased over the past decades, but this is due to successes in conventional breeding, not GM traits.

Neither have GM crops decreased pesticide use. The adoption of GM Bt maize and cotton has resulted in a slight decrease in the volume of insecticide sprays, but this decrease is likely to be unsustainable as pests gain resistance to the Bt toxins and secondary pests take over. Also, the reduction in insecticidal sprays is dwarfed by the massive increase in herbicide use caused by the adoption of GM herbicide-tolerant crops. The adoption of these GM crops has caused farmers to spray 383 million more pounds (174 million kg) of herbicides than they would have done in the absence of GM herbicide-tolerant seeds.

This increase is largely due to the spread of weeds resistant to glyphosate, the herbicide most commonly used on GM crops. As a "solution" to the problem of glyphosate-resistant weeds, biotech companies have developed crops engineered to tolerate several different herbicides, including potentially even more toxic herbicides such as dicamba and 2,4-D (an extremely toxic ingredient of Agent Orange). The resulting chemical treadmill only benefits the GM seed companies, which profit from each failure of their technologies because the failure creates a new opportunity for them to sell more chemicals in increasingly complex mixtures. Claims for the environmental friendliness of the no-till farming system as practised with GM herbicide-tolerant crops are also unjustified.

Glyphosate over-use is also causing other problems for farmers, such as reducing crop vigour by making soil nutrients unavailable to crops and causing or exacerbating plant diseases that impact yield. Manufacturer claims that glyphosate/Roundup is an environmentally benign herbicide with low toxicity have proved to be false, with a growing number of studies showing that it persists in the environment and has toxic effects, in addition to studies showing that it is toxic to humans and causes birth defects and cancer.

Claims of reductions in insecticide use through Bt crops are suspect when it is considered that the entire GM plant is an insecticide. Also, Bt crop technology is being undermined by the

emergence of resistant and secondary pests, which force farmers to go back to spraying complex and expensive chemical cocktails. And the increased use of insecticidal seed treatments on GM and non-GM seed alike raises the possibility that insecticide use has not been reduced through Bt crops but that it is simply less visible to farmers and consumers.

Statements that the Bt toxin in Bt crops only affects insect pests have been shown to be false by studies showing negative effects on a wide range of organisms, including beneficial insects that help protect crops and beneficial soil organisms that enhance crop growth and health.

Economic impacts of GM crops on farmers appear to be variable. Reports have emerged of escalating prices for GM seeds and the chemicals they are engineered to depend on. This pattern is enabled by the consolidation of the seed market under the control of the GM and agrochemical industry and the absence of real competition.

At odds with claims that GM crops increase farmer choice, in reality their introduction marks the disappearance of farmer choice due to two mechanisms. First, as the GM industry gains control over the seed market in a region, desirable non-GM seed varieties are pulled from the market. Second, the biotech industry lobbies for "freedom of choice" for farmers, claiming that GM and non-GM crops (including organic) can "co-exist". This opens the door for GM crops, causing farmers who wish to grow non-GM or organic crops to lose their freedom of choice due to GM contamination. Time and again, this has resulted in lost markets and increased costs to farmers and the food and feed industry.

GM traits can spread to other crops, wild plants, and other unrelated species by horizontal gene transfer (HGT) through several mechanisms, some of which are more likely than others. The potential consequences of HGT have not been adequately considered by regulators.

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6. CLIMATE CHANGE AND ENERGY USE

Climate change is often used as a reason to claim that we need GM crops.[1] But the evidence suggests that the solutions to climate change do not lie in GM. This is because tolerance to extreme weather conditions such as drought and flooding – and resistance to the pests and diseases that often accompany them – are complex traits that cannot be delivered through GM.

Where a GM crop is claimed to possess such complex traits, they have generally been achieved through conventional breeding, not GM. Simple GM traits such as pest resistance or herbicide tolerance are added to the conventionally bred crop so as to put the biotech company's "brand" on it after the complex trait is developed through conventional breeding.

While the resulting crop is often claimed as a GM success, this is untrue. It is a success of conventional breeding, with added GM traits. The GM traits do not contribute to the agronomic performance of the crop but make the crop the property of a biotech company and (in the case of herbicide tolerance) keep farmers dependent on chemical inputs sold by the same company.

Section at a glance

- GM will not solve the problems of climate change. Tolerance to extreme weather conditions involves complex, subtly regulated traits that genetic engineering is incapable of conferring on plants.
- Most GM crops depend on large amounts of herbicides, which in turn require large amounts of fossil fuels in manufacture.
- No GM nitrogen-use-efficient crops have been successfully commercialised even though promoters of the technology have been promising them for more than a decade.
- Conventional breeding is far ahead of GM in developing climate-ready and nitrogen-use-efficient crops.
- Additional means to cope with climate change include the many locally-adapted seeds conserved by farmers across the world and agroecological soil, water, and nitrogen management systems.

6.1 **Myth:** GM will deliver climate-ready crops **Truth:** Conventional breeding outstrips GM in delivering climate-ready crops

In December 2011 the US Department of Agriculture (USDA) deregulated Monsanto's drought-tolerant maize variety MON87460.[2] It was hailed as the first commercialised GM crop designed to resist stressful environmental conditions like drought. But the USDA, in its assessment of the crop, noted that many non-GM maize varieties on the market are at least as effective as Monsanto's engineered maize variety in managing water use. "The reduced yield [trait] does not exceed the natural variation observed in regionally-adapted varieties of conventional corn," USDA said, adding, "Equally comparable varieties produced through conventional breeding techniques are readily available in irrigated corn production regions."[3]

This is to be expected, given that GM crops are developed by adding GM traits to the best existing conventionally bred varieties.

Meanwhile, conventional breeding, sometimes helped by marker assisted selection, has outstripped GM in producing numerous climate-ready crops. Examples include:

- Maize varieties that yield well in drought conditions,[4] including some developed for farmers in Africa[5,6,7]
- Cassava that gives high yields in drought conditions and resists several diseases[8]
- Climate-adapted, high-yield sorghum varieties developed for farmers in Mali[9]
- Beans resistant to heat, drought, and disease[10,11]
- Pearl millet, sorghum, chickpea, pigeon pea and groundnut varieties that tolerate drought and high temperatures[12]
- Rice varieties bred to tolerate drought, flood, disease, and saline (salty) soils[13]
- Flood-tolerant rice varieties developed for Asia[14,15]
- Over 2,000 indigenous rice varieties that are adapted to environmental fluctuations, as well as varieties that resist pests and diseases, registered by Navdanya, a seed-keeping NGO based in India[16]
- Tomato varieties developed by Nepali farmers that tolerate extreme heat and resist disease.[17]

It should be borne in mind that only a part of the solution to climate change lies in plant genetics. Insofar as genetics is the solution, humanity will continue to rely on the same source that GM seed companies mine for their germplasm – the hundreds of thousands of locally adapted seed varieties developed and conserved over centuries by farmers worldwide. These varieties are our living germplasm bank.

The part of the solution that lies beyond plant genetics will be found in proven effective agroecological farm management techniques, such as building organic matter into the soil to conserve water, planting a diversity of crops, rotating crops, and choosing the right plant for the conditions.

6.2 **Myth:** No-till farming as practised with GM crops is climate-friendly as it sequesters more carbon
Truth: No-till farming does not sequester more carbon

Chemically-based agriculture is a major contributor to climate change, producing over 20% of greenhouse gas emissions.[18] GM proponents claim that GM crops can help reverse this trend by enabling the adoption of no-till farming, which avoids ploughing and relies on herbicide applications to control weeds. GM proponents argue that no-till sequesters (stores) more carbon in the soil than ploughing, preventing the carbon from being released into the atmosphere as the greenhouse gas carbon dioxide.

On the basis of this argument, Monsanto is lobbying for GM Roundup Ready crop cultivation to be made eligible for carbon credits under the United Nations' Clean Development Mechanism (CDM).[19] The CDM aims to promote technologies that mitigate climate change. Industrialized countries and companies in the Global North can continue to emit the same amount of greenhouse gases and still meet their required emissions reductions by funding CDM projects, most of which are in the Global South.

If Monsanto succeeds in its lobbying and farmers that grow Roundup Ready crops can access carbon credits for no-till, then sales of Monsanto's seeds and agrochemicals will increase, as governments will encourage farmers to plant Roundup Ready crops to qualify for carbon credits.

But industry claims of improved carbon sequestration for GM Roundup Ready crops with no-till are not supported by research. A comprehensive review of the scientific literature found that no-till fields sequester no more carbon than ploughed fields when carbon sequestration at soil depths greater than 30 cm is taken into account. Studies claiming to find carbon sequestration benefits from no-till only measure carbon sequestration down to a depth of about 30cm and so do not give an accurate picture.[20]

6.3 **Myth:** GM will solve the nitrogen crisis **Truth:** GM has not delivered nitrogen-efficient crops

Synthetic nitrogen fertilizer is used in GM farming, as in all chemically-based agriculture. There are many problems associated with its production and use. The production process uses large amounts of natural gas, a non-renewable fossil fuel.[21] A UK study found that nitrogen fertilizer production can account for more than 50% of the total energy used in agriculture.[22]

Nitrogen fertilizer produces greenhouse gases at the time of manufacture and again when used on fields,[22] giving off nitrous oxide, a greenhouse gas 300 times more potent than carbon dioxide.[23] Fertilizer-intensive agriculture is the largest source of human-created nitrous oxide emissions in the US[24] and will be a major source in any country using chemically-based agriculture.

The profitability of farming is highly dependent on the cost of fertilizers, and the cost of nitrogen fertilizer is tied to natural gas prices.[21] In Canada, a major producer, the price of nitrogen fertilizer reached a record high in 2008.[25] According to some analysts, peak gas, the point at which the maximum rate of gas extraction is reached and supplies enter terminal decline is expected to arrive around 2020.[26] As this point gets closer, prices will rise. Already the industry is ramping up expensive and environmentally damaging strategies, like fracking, for natural gas extraction.

For these reasons, agriculture cannot continue to depend on synthetic nitrogen fertilizer. Other ways of managing nitrogen must be found.

Some plants, including most legumes (the bean family of plants, which includes soy and peanuts), fix nitrogen directly from the air with the help of nitrogen-fixing bacteria. But other crops, such as wheat and barley, cannot do this and need to be fed nitrogen through the soil.

Proponents claim that genetic engineering can produce crops with high nitrogen use efficiency (NUE) that require less nitrogen fertilizer.

But GM technology has not produced any commercially available NUE crops.[27] On the other hand, conventional breeding has successfully delivered improvements in NUE in a number of crops. Estimates for wheat from France show an increase in NUE of 29% over 35 years, and Mexico has improved wheat NUE by 42% over 35 years.[27]

Studies show that organic, low-input and sustainable farming methods are the key to nitrogen management. One study calculated the potential nitrogen production by such methods to be 154 million tonnes, a potential which far exceeds the nitrogen production from fossil fuel.[28]

Sustainable nitrogen management methods include the planting of legumes in rows between the main crop, or in a crop rotation. This makes growth-promoting nitrogen available to other plants growing nearby at the same time or planted in subsequent cropping seasons.

Study findings include:

- Planting legumes on degraded land in Brazil successfully fixed nitrogen in soil, restoring soil and ecosystem biodiversity in the process.[29]
- Maize/peanut intercropping (growing two or more crops in close proximity) increased soil nitrogen and nutrients, increased growth of beneficial soil bacteria, and was expected to promote plant growth, as compared with monoculture, in experiments in China.[30]
- Planting legume cover crops (crops planted to preserve soil) could fix enough nitrogen to replace the amount of synthetic fertilizer currently in use, according to data from temperate and tropical agroecosystems.[28]

Agroecological methods of managing nitrogen solve another major problem associated with the application of synthetic nitrogen fertilizer – loss of soil nitrogen though agricultural runoff. In the runoff process, nitrogen leaches from soil in the form of nitrate, polluting groundwater. It can get into drinking water, threatening human and livestock health.

Agroecological, organic, low-input, and sustainable farming practices have been found to reduce soil nitrogen losses in the form of nitrate by 59–62% compared with conventional farming practices.[31] The result is reduced nitrate pollution and better conservation of nitrogen in soil.

6.4 **Myth:** GM crops reduce energy use **Truth:** GM crops are energy-hungry

> *"We have tried to have more efficient farming, with fewer people, more machines and a greater dependency on pesticides, fertilizers, GM crops and energy, using 10 kilocalories to produce one kilocalorie [of food delivered to the consumer]. But that is only possible if there is cheap oil. The system basically is bankrupt, which is why we need to change it to a more modern, advanced system, which will create energy, rather than consume it, and is not dependent on fossil energy, but more on people and better science."*
>
> – Hans Herren, development expert and co-chair, International Assessment of Agricultural Knowledge, Science and Technology, (IAASTD), a three-year project on the future of farming involving more than 400 experts from across the world[32]

In the US food system, 10 kilocalories of fossil energy are required for every one kilocalorie of food delivered to the consumer.[33] Two-thirds of that energy goes into producing synthetic fertilizers and on-farm mechanisation.[34]

There is widespread agreement that the energy consumption of agriculture must be radically reduced. GM proponents claim that GM crops can help in that process. As evidence they cite a report by Graham Brookes and Peter Barfoot, directors of PG Economics, a consultancy firm to the agrochemical and biotech industry.[35,36]

Brookes and Barfoot offer as a major reason for this claimed reduction in energy use the no-till farming method that is used in the cultivation of GM Roundup Ready crops. The idea is that no-till reduces the number of tractor passes that farmers have to make across their fields in ploughing.

But data from Argentina comparing the energy used in growing GM Roundup Ready soy and non-GM soy showed that, while no-till did reduce farm operations (tractor passes across the field), the production of GM soy required more energy in both no-till and tillage systems. The reason for the increase was the large amount of energy consumed in the production of herbicides (mostly Roundup) used on GM soy.[37]

Proven methods of reducing the amount of fossil energy used in farming include minimising the use of synthetic pesticides and fertilizers, selecting farm machinery appropriate for each task, limiting irrigation, and using agroecological techniques to manage soil fertility and control pests.[33]

Organic farming systems use just 63% of the energy required by chemically-based farming systems, largely because they eliminate the energy required to produce nitrogen fertilizer and pesticides.[38]

Organic, low-input, and agroecological farming is well suited to the Global South. A study in Ethiopia, part-funded by the UN Food and Agriculture Organisation (FAO), showed that compost can replace chemical fertilizers and that it increased yields by more than 30%. The crops had better resistance to pests and disease and there were fewer difficult weeds.[39]

6.4.1. Peak oil and gas make GM crops redundant

According to some analysts, peak oil – the point when the maximum rate of extraction is reached, after which production goes into terminal decline – has already arrived. Peak gas is expected around 2020.[26] Peak oil and gas mark the end of chemically-based agriculture because nitrogen fertilizers are synthesised using large amounts of natural gas, and pesticides (including herbicides) are made from oil.

GM firms constantly promise new crops that are not reliant on the chemical model of farming. But GM seeds are created by agrochemical companies and are heavily dependent on pesticides and fertilizers. According to industry data, two-thirds of GM crops worldwide are herbicide-tolerant[40] – in other words, they are designed to rely on high doses of herbicide. Many of the newest GM crops are engineered to tolerate several different herbicides (see section 5).

Agriculture cannot continue to depend on non-renewable and increasingly expensive external inputs. Future food production will reduce or eliminate pesticide use and rely on renewable biologically-based fertilizers – such as compost and animal manure – produced on the farm or locally.

Conclusion to Section 6

GM crops offer no effective or sustainable solutions to climate change. Tolerance to extreme weather conditions is a complex trait that cannot be inserted into plants through genetic engineering. Most GM crops planted worldwide depend on large amounts of herbicides, which in turn require large amounts of fossil fuels in manufacture. GM crops, like all chemically-farmed crops, also depend on energy-hungry and greenhouse-gas-emitting nitrogen fertilizer. No GM nitrogen-use-efficient crops are available on the market.

In contrast, conventional breeding, sometimes helped by marker assisted breeding, is far ahead of GM in developing climate-ready and nitrogen-use-efficient crops. Additional means to cope with climate change include the many locally-adapted seeds conserved by farmers across the world and agroecological soil, water, and nitrogen management systems.

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7. FEEDING THE WORLD

7.1 **Myth:** GM crops are needed to feed the world's growing population
Truth: GM crops are irrelevant to feeding the world

"We strongly object that the image of the poor and hungry from our countries is being used by giant multinational corporations to push a technology that is neither safe, environmentally friendly nor economically beneficial to us. We do not believe that such companies or gene technologies will help our farmers to produce the food that is needed in the 21st century. On the contrary, we think it will destroy the diversity, the local knowledge and the sustainable agricultural systems that our farmers have developed for millennia, and that it will thus undermine our capacity to feed ourselves."

– Statement signed by 24 delegates from 18 African countries to the United Nations Food and Agricultural Organization, 1998

"If anyone tells you that GM is going to feed the world, tell them that it is not... To feed the world takes political and financial will."

– Steve Smith, head of GM company Novartis Seeds UK (now Syngenta), public meeting on proposed local GM farm scale trial, Tittleshall, Norfolk, UK, 29 March 2000

GM crops are promoted as a way of solving world hunger at a time when the population is expected to increase. But it is difficult to see how GM can contribute to solving world hunger when there are no GM crops available that increase intrinsic yield (see Section 5). Nor are there any GM crops that are better than non-GM crops at tolerating poor soils or challenging climate conditions.

Instead, most currently available GM crops are engineered for herbicide tolerance or to contain a pesticide, or both. The two major GM crops, soy and maize, mostly go into animal feed, biofuels to power cars, and processed human food – products for developed nations that have nothing to do with meeting the basic food needs of the poor and hungry. GM corporations are answerable to their shareholders and thus are interested in profitable commodity markets, not in feeding the poor and hungry.

Even if a GM crop did appear that gave higher yields than non-GM crops, this would not impact

Section at a glance

- GM crops are promoted as necessary to feed the world's growing population. But it seems unlikely that they could make a significant contribution as they do not deliver higher yields or produce more with less inputs than non-GM crops.
- Most GM crops are engineered to tolerate herbicides or to express a pesticide – properties that are irrelevant to solving hunger.
- Hunger is not caused by a lack of food in the world. It is a problem of distribution and poverty, which GM cannot solve.
- The IAASTD report, authored by over 400 international experts, concluded that the key to food security lay in agroecological farming methods. The report did not endorse GM, noting that yields were "variable" and that better solutions were available.
- Agroecological farming has resulted in significant yield and income benefits to farmers in the Global South, while preserving soil for future generations.
- GM is not needed to feed the world. Conventional plant breeding has already delivered crops that are high-yielding, disease- and pest-resistant, tolerant of drought and other climatic extremes, and nutritionally enhanced – at a fraction of the cost of GM.

the problem of hunger. This is because the root cause of hunger is not a lack of food, but a lack of access to food. According to the UN Food and Agriculture Organisation, we already produce more than enough food to feed the world's population and could produce enough with existing agricultural methods to feed 12 billion people.[1] The problem is that the poor have no money to buy food and increasingly, no access to land on which to grow it. Hunger is a social, political, and economic problem, which GM technology cannot address. GM is a dangerous distraction from real solutions and claims that GM can help feed the world can be viewed as exploitation of the suffering of the hungry.

7.1.2. GM crops for Africa: Catalogue of failure

A handful of GM crops have been promoted as helping small-scale and poor farmers in Africa. However, the results were the opposite of what was promised.

GM sweet potato yielded poorly, lost virus resistance

The virus-resistant sweet potato has been a GM showcase project for Africa, generating global media coverage. Florence Wambugu, the Monsanto-trained scientist fronting the project, has been proclaimed an African heroine and the saviour of millions, based on her claims that the GM sweet potato doubled output in Kenya. Forbes magazine even declared her one of a tiny handful of people around the globe who would "reinvent the future".[2]

But it eventually emerged that the claims being made for the GM sweet potato were untrue, with field trial results showing it to be a failure. The GM sweet potato was out-yielded by the non-GM control and succumbed to the virus it was designed to resist.[3,4]

In contrast, a conventional breeding programme in Uganda produced a new high-yielding variety that was virus-resistant and raised yields by roughly 100%. The Ugandan project achieved its goal in a fraction of the time and cost of the GM project. The GM sweet potato project, over 12 years, consumed funding from Monsanto, the World Bank, and USAID to the tune of $6 million.[5]

GM cassava lost virus resistance

The potential of genetic engineering to boost the production of cassava – one of Africa's staple foods – by defeating a devastating virus has been heavily promoted since the mid-1990s. It was even claimed that GM cassava could solve hunger in Africa by increasing yields as much as tenfold.[6]

But almost nothing appears to have been achieved. Even after it became clear that the GM cassava had suffered a major technical failure, losing resistance to the virus,[7] media stories continued to appear about its curing hunger in Africa.[8,9]

Meanwhile, conventional (non-GM) plant breeding has quietly produced a virus resistant cassava that is already proving successful in farmers' field, even under drought conditions.[10]

Bt cotton failed in Makhatini

> *"The [GM cotton] seed itself is doing poorly. Without irrigation, and with increasingly unpredictable rain, it has been impossible to plant the cotton. In 2005 T. J. Buthelezi, the man whose progress was hymned by Monsanto's vice-president not three years before, had this to say: 'My head is full – I don't know what I'm going to do. I haven't planted a single seed this season. I have paid Rand 6,000 (USD 820, GBP 420) for ploughing, and I'm now in deep debt.' T. J. is one of the faces trucked around the world by Monsanto to prove that African farmers are benefiting from GM technology."*
>
> – Raj Patel, "Making up Makhatini", in Stuffed and Starved[11]

Makhatini in South Africa was home to a showcase GM Bt cotton project for small-scale farmers. The project began with 3000 smallholder farmers cultivating Monsanto's Bt cotton between 1998 and 2001,[12] with over 100,000 hectares planted. By 2002, the area planted had crashed to 22,500 hectares, an 80% reduction in four years.[13,11]

A 2003 report on the project calculated that crop failures left the farmers who had adopted the expensive Bt cotton with debts of $1.2 million.[5] A separate study concluded that the project did not generate sufficient income to generate a "tangible and sustainable socioeconomic improvement".[14]

By 2004, 85% of farmers who used to grow Bt cotton had given up. The farmers found pest problems and no increase in yield. Those farmers who still grew the crop did so at a loss. They continued only because the South African government subsidised the project from public funds; the company that sold the cottonseed and bought the cotton was their only source of credit; and there was a guaranteed market for the cotton.[13,11]

A 2012 review reported that by the 2010/11 growing season, the area planted to Bt cotton had shrunk to a minuscule 500 hectares – a decline of more than 90% from the area under cultivation during the period of Bt cotton's claimed success (1998–2000). Yields continued to vary widely according to rainfall levels, hovering within 10% of what they were before Bt cotton was introduced. Overall pest control costs remained significantly higher with Bt cotton (65% of total input costs) than with non-Bt cotton (42% of total input costs).

The review concluded that the main value of Makhatini project appears to have been as a public relations exercise for GM proponents, providing "crucial ammunition to help convince other African nations to adopt GM crops" and that there was a "disconnect" between how the project was represented and "the realities faced by its cotton growers".[12]

GM soy and maize project ends in ruin for poor farmers

A GM soy and maize farming project ended in disaster for poor black farmers in South Africa. The Eastern Cape government was criticised for its support of this so-called "Green Revolution" project, which was launched in 2003–2004. A research study by the Masifunde Education and Development Project Trust, together with Rhodes University, found that the programme had disastrous results for farmers.

"We saw a deepening of poverty and people returning to the land for survival," said Masifunde researcher, Mercia Andrews. The study raised concerns about feeding schemes conducted on animals with "alarming results", including damage to internal organs. It presented evidence of weed and pest problems, contamination of crops with GM pollen, and the control exercised by big companies over local and global food systems as a result of patented seeds.[15]

We conclude from these examples that it is irresponsible to pressure poor farmers in the Global South into gambling their farms and livelihoods on risky GM crops when proven effective alternatives exist.

> *"To feed 9 billion people in 2050, we urgently need to adopt the most efficient farming techniques available. Today's scientific evidence demonstrates that agroecological methods outperform the use of chemical fertilizers in boosting food production where the hungry live – especially in unfavorable environments.*
>
> *"To date, agroecological projects have shown an average crop yield increase of 80% in 57 developing countries, with an average increase of 116% for all African projects. Recent projects conducted in 20 African countries demonstrated a doubling of crop yields over a period of 3–10 years.*
>
> *"Conventional farming relies on expensive inputs, fuels climate change and is not resilient to climatic shocks. It simply is not the best choice anymore today.*
>
> *"Agriculture should be fundamentally redirected towards modes of production that are more environmentally sustainable and socially just."*
>
> – Olivier De Schutter, UN special rapporteur on the right to food and author of the report, "Agroecology and the right to food"[32,33]

7.1.3. The biofuels boom and the food crisis

> *"The agribusiness giants who have developed and patented genetically modified crops have long argued that their mission is to feed the world, rarely*

missing an opportunity to mention starving Africans. Their mission is, in fact, to make a profit.

"Land rights for small farmers, political stability, fairer markets, education and investment hold the key to feeding Africa but offer little prospect of increased profits.

"The climate crisis was used to boost biofuels, helping to create the food crisis; and now the food crisis is being used to revive the fortunes of the GM industry."

– Daniel Howden, Africa correspondent, The Independent (UK)[16]

"The cynic in me thinks that they're just using the current food crisis and the fuel crisis as a springboard to push GM crops back on to the public agenda. I understand why they're doing it, but the danger is that if they're making these claims about GM crops solving the problem of drought or feeding the world, that's bullshit."

– Denis Murphy, head of biotechnology, University of Glamorgan, Wales[17]

The 2007–2008 global food crisis led to food riots around the world, as the escalating price of staple crops pushed food out of reach of the poor and hungry. The crisis is ongoing – in early 2011 global food prices remained close to their 2008 peak.[18] They declined 8% between September and December 2011, though the World Bank reported that they were still high, with the 2011 annual food price index exceeding the 2010 annual index by 24%.[18]

GM proponents have used the food crisis to claim that anti-GM activists in the Global North are keeping the Global South hungry by creating unfounded fears about GM crops. These high-technology GM crops, they claimed, could help solve the hunger problem, if only the activists in affluent countries would stop interfering. But the World Bank and the United Nations Food and Agriculture Organisation identified the biofuels boom – not a lack of GM foods – as the main cause of the 2007–2008 food crisis.[19,20]

Biofuels are crops used for fuel. Vast tracts of cropland have been taken out of food production to grow biofuels for cars, funded by generous government subsidies. This has made food scarcer, pushing up costs.

An added factor is that the growth of the biofuels industry has created a link between agriculture and fuel that never existed before.

"A key question for our scientists, and politicians to address, and to have the courage to demand that industry addresses it too, is whether GM technology can and will co-exist in the global agricultural toolbox with other technologies, without destroying those other tools. Apart from more promise than delivery, and delivery of only private benefits like greater market share for their own chemical pesticides, GM has brought with it a marked narrowing of seed varieties available to farmers, a concentration of ownership of seed production and sales, and a concentration in ownership and control of the knowledge (intellectual property rights or IPRs) required for agricultural production.

"In 2002, the director of the Vietnamese government agricultural research centre told me at a conference in Asia that he could spend all of his annual R&D budget (US$20m, as I recall) just on lawyers, trying to sort out what materials his researchers could and could not use, and on licence fees for such IPRs, according to the intellectual property rights jungle which has grown on plant and crop materials and molecules. Is this kind of commercial restriction, and narrowing of diversity of agricultural innovation trajectories, helping such food-poor countries to gain food security?

"This concentration and narrowing, and the associated transformation of agriculture into industrialised monocrop production requiring more expensive and unsustainable inputs, which in turn ignores and externalises entirely predictable pest and weed resistance and thus short-term yield drops, cannot be a sustainable technology. Nor does it seem that it could co-exist with other technologies in the so-called toolbox."

– Professor Brian Wynne, ESRC Centre for Economic and Social Aspects of Genomics, Cesagen Lancaster University, UK[43]

Previously, agricultural markets were driven only by food demands and were not linked to petroleum markets. But now they are tightly linked, because agriculture provides the crops that are used to make the biofuels alternative to petrochemical fuels. Four major food and feed crops – sugarcane, maize, wheat, and soy – are now used for biofuels feedstock. So the biofuels boom has coupled food prices to fossil fuel prices,[18] with the result that food prices will continue to spiral as petroleum becomes scarcer and more expensive.

The same companies that produce GM seeds also produce feedstocks for biofuels. This shows that these companies are not motivated by a desire to feed the world but by a desire to make a profit.

7.1.4 Food speculation and hunger

An additional cause of the 2007–2008 food crisis (apart from the rush to biofuels) was financial speculation in food commodity markets. This ongoing trend drives up prices for the crops that are traded internationally on a large scale, namely maize, wheat, and soy. One report on the topic concluded, "Food markets should serve the interests of people and not those of financial investors... Given that hunger still exists in the world, even small price increases that are driven by financial investment are scandalous. We must not allow food to become a purely financial asset."[21]

GM crops do not provide a solution to the problem of financial speculation in food markets.

7.2 **Myth:** GM crops are vital to achieve food security
Truth: Agroecological farming is the key to food security

> *"Agroecology mimics nature not industrial processes. It replaces the external inputs like fertilizer with knowledge of how a combination of plants, trees and animals can enhance productivity of the land. Yields went up 214% in 44 projects in 20 countries in sub-Saharan Africa using agroecological farming techniques over a period of 3 to 10 years... far more than any GM crop has ever done."*
>
> – Olivier De Schutter, UN special rapporteur on the right to food[22]

In 2008 the World Bank and four United Nations agencies completed a four-year study on the future of farming. Conducted by over 400 scientists and experts from 80 countries and endorsed by 62 governments, the International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD) report did not endorse GM crops as a solution to world hunger. The report pointed out that yields of GM crops were "highly variable", providing "yield gains in some places and yield declines in others".[23]

The IAASTD identified agroecological farming as the key to future food security. The report called for more cooperation between farmers and interdisciplinary teams of scientists to build culturally acceptable and sustainable food production systems.[23] Examples of such systems documented in IAASTD and other sources include:

- Low-input, energy-saving practices that preserve and build soil, conserve water, and enhance natural pest resistance and resilience in crops
- Innovative farming methods that minimize or eliminate costly chemical pesticides and fertilizers
- Use of thousands of traditional varieties of major food crops which are naturally adapted to stresses such as drought, heat, harsh weather conditions, flooding, salinity, poor soil, and pests and diseases[24]
- Programmes that enable farmers to cooperatively preserve and improve traditional seeds
- Use of existing crops and their wild relatives in traditional breeding programmes to develop varieties with useful traits
- Use of safe techniques of modern biotechnology, such as marker assisted selection (MAS), to speed up traditional breeding. Unlike GM technology, MAS can produce new varieties of crops with valuable genetically complex properties such as enhanced nutrition, taste, high yield, resistance to pests and diseases, and tolerance to drought, heat, salinity, and flooding.[25]

Sustainable agriculture projects in the Global South have produced dramatic increases in yields and food security.[26,27,28,29,30,31] A 2008 United Nations report looked at 114 farming projects in 24 African countries and found that organic or near-organic practices resulted in yield increases averaging over 100%. In East Africa, a yield increase of 128% was found. The report concluded that organic agriculture can be more conducive to food security in Africa than chemically-based production systems, and that it is more likely to be sustainable in the long term.[29]

These results serve as a reminder that plant genetics are only a part of the answer to food security. The other part is how crops are grown. Sustainable farming methods that preserve soil and water and minimize external inputs not only ensure that there is enough food for the current population, but that the land stays productive for future generations.

7.2.1. Small farms are more efficient

Research confirms that future food security lies in the hands of small farmers. Small farms are more efficient than large ones, producing more crops per hectare of land.[34,35,36,37]

7.2.2. Sustainable agriculture can reduce poverty

Studies based in Asia, Africa, Latin America

and the Caribbean have found that organic and agroecological farming can combat poverty in an environmentally sustainable way:

- Farmers growing organic crops for export and domestic markets in Latin America and the Caribbean had higher incomes than a control group of farmers using chemically-based methods. Reasons included the lower cost of organic technologies; the substitution of labour and organic inputs for more expensive chemical inputs that often require access to credit; premiums paid for organic products; and the strong long-term relationships that organic farmers developed with buyers, which resulted in better prices. As a bonus, organic production was associated with positive effects on the health of farm workers. Concern about pesticide poisoning was an important factor in farmers' adoption of organic farming.[38]
- The income of farmers in China and India improved after they switched to organic systems and was greater than that of farmers using chemically-based methods. The study concluded that the promotion of organic agriculture among small farmers can contribute to poverty alleviation.[39]
- Certified organic farms in tropical Africa involved in production for export were more profitable than those involved in chemically-based export production. The result was decreased poverty and increased food security for farming communities, as people had more money to buy food. Also, organic conversion brought increases in yield.[40]
- Organic systems in Africa were found to raise farm incomes as well as agricultural productivity. Reasons for the higher incomes included lower input costs, as expensive synthetic pesticides and fertilizers were not used; and use of local, inexpensive, and readily available technologies.[29]
- The agroecological "integrated rice-duck" system of using ducks and fish to control pests in rice paddies in Japan, China, India, the Philippines, and Bangladesh has cut labour costs for weeding, reduced pesticide costs, increased yields by up to 20%, and boosted farm incomes by up to 80%.[41,42]

7.2.3. Who owns food?

Traditionally, most food crop seeds have not been owned by anyone. Farmers have been free to save seeds from one year's crop for the next year's crop. Around 1.4 billion farmers in the Global South rely on such farm-saved seed for their livelihoods.[44]

But this ancient practice is being undermined. The transgenes used in creating GM crops are patented and owned by GM companies. The patents forbid farmers from saving seed to plant the following year. They have to buy new seed each year.

While an increasing number of non-GM seeds are also being patented (in many cases by the big GM companies such as Monsanto, Dupont, and Syngenta), GM seeds are easier to patent as the artificial genetic constructs can be more clearly identified and there are fewer legal "grey areas".[45] So for the time being, at least, GM will remain the technology of choice for the seed multinationals.

In the United States and Canada, the presence of a company's patented GM genes in a farmer's harvest has been used by GM companies, particularly Monsanto, as the basis for litigation against the farmer. Contamination from cross-pollination happens readily, so the harvests of many farmers who have not planted Monsanto seed have tested positive for GM genes and Monsanto has sued them for patent infringement. This has pushed many farmers into switching to buying Monsanto's seed, because then they are safer from litigation. Farmers' claims that they have not intentionally planted GM crops have not protected them from having to pay large cash settlements or damages as a result of civil lawsuits.[46]

Patented GM seeds transfer control of food production from farmers to seed companies. GM companies co-opt centuries of farmer knowledge that went into creating locally adapted and genetically diverse seed stocks by adding one GM gene on top of the collective creation of generations of farmers.

Patents also transfer control of the food supply from the Global South to developed countries in the Global North. This is because most of the world's genetic resources for food crops are in the South, whereas most patents are held in

the North.[47] There is widespread concern in the Global South about the "biopiracy" of its genetic resources by the Global North, involving seed patenting and the loss of farmers' rights to save seed.

Some GM proponents have called for GM crops to be developed through public funds for the benefit of humanity.[48] But it is difficult to justify gambling taxpayer funds on speculative GM "solutions" to problems that can be solved using methods that are simpler, cheaper, and available now. Nor would any public or private entity have an incentive to fund the lengthy and expensive process of GM crop development unless they owned a patent that would enable them to recoup their expenses and make a profit.

Patents have no place in the agricultural system. To protect the security of the food supply and to ensure food sovereignty for each nation, governments must establish policies that ensure that the control of food production remains in the hands of farmers.

7.3 **Myth:** GM is needed to provide the crops that will enable us to survive the challenges ahead
Truth: Non-GM breeding methods are more effective at creating crops with useful traits

> *"The advantage of science is not in principle, for its own self – it's because it does something useful and valuable, that people want. If it is not supporting those particular objectives, I think we should take a much more sceptical view of it."*
>
> – Michael Meacher, UK environment minister 2001–2003[49]

When people hear about "supercrops" such as flood-tolerant rice, drought-tolerant maize, salt-tolerant wheat, pest-resistant chickpeas, low-allergen peanuts, iron-rich beans, beta-carotene-enriched cassava, and heart-healthy soybeans, many automatically think of GM.

But all these improved crops were created without GM. They are the products of conventional (natural) breeding, in some cases helped by marker assisted selection, or MAS. MAS, sometimes called precision breeding, is a largely uncontroversial branch of biotechnology that can speed up conventional breeding by identifying genes linked to important traits. MAS does not involve inserting foreign genes into the DNA of a host plant and avoids the risks and uncertainties of genetic engineering. It is widely supported by environmentalists and organic farming bodies.

Conventional breeding and MAS have succeeded where GM has failed in developing crops with useful traits such as tolerance to extreme weather conditions and poor soils, disease resistance, and enhanced nutritional value. Such traits are known as complex traits because they involve many genes working together in a precisely regulated way. Only conventional breeding methods, sometimes helped by MAS, are able to produce crops with the desired complex traits. In contrast, GM technology can only manipulate one or a few genes at a time and is unable to confer precise and integrated control of expression of GM genes. Therefore it is incapable of producing crops with desired complex traits that rely on multiple genes working together.

Conventional breeding and MAS use the many existing varieties of crops to create a diverse, flexible, and resilient crop base. GM technology offers the opposite – a narrowing of crop diversity and an inflexible technology that requires years and millions of dollars in investment for each new trait.[50,51]

Non-GM breeding successes usually gain minimal media coverage, in contrast with the often speculative claims of potential GM "miracles". Thanks to the huge public relations budgets of biotechnology companies, these claims are broadcast far and wide – but have little grounding in fact.

7.3.1. The GM successes that never were

Many crops developed through conventional breeding and marker-assisted selection (MAS) are wrongly claimed as GM successes. These fall into three broad categories:

Conventionally bred crop with GM tweak

> *"Biotech traits by themselves are absolutely useless unless they can be put into the very best germplasm."*
>
> – Brian Whan, spokesman for Monsanto subsidiary InterGrain[52]

Typically, GM firms use conventional breeding, not GM, to develop crops with traits such as drought tolerance or disease resistance. They first obtain germplasm from the best varieties developed over years by farmers and breeders. Then they use conventional breeding and MAS to achieve the desired complex trait. Finally, once they have developed a successful variety using conventional breeding, they use GM to engineer

in the company's proprietary genes, so that they can patent and own the crop. This GM tweak, often a herbicide-tolerant or insecticidal gene, adds nothing to the agronomic performance of the crop.

This process was mentioned in a news broadcast about Monsanto's 2010 buy-out of part of a Western Australia cereal breeding company, InterGrain. An InterGrain spokesman explained Monsanto's interest in his company: "A really important concept is that biotech traits by themselves are absolutely useless unless they can be put into the very best germplasm."[52]

An example of a GM product developed in this way is Monsanto's VISTIVE® soybean, which has been described as the first GM product with benefits for consumers. These low linolenic acid soybeans were designed to produce oil that would reduce unhealthy trans fats in processed food made from the oil. They were created by conventional breeding. But Monsanto turned them into a GM crop by adding a GM trait – tolerance to its Roundup herbicide.[53]

Interestingly, Iowa State University developed some even lower linolenic acid soybean varieties than the VISTIVE and did not add any GM traits to them.[54] Very little has been heard about them, compared with VISTIVE.

Another product of this type is Syngenta's Agrisure Artesian drought-tolerant maize. The crop was developed using non-GM breeding, but herbicide tolerant and insecticidal transgenes were subsequently added through genetic engineering.[55]

Conventionally bred crop without GM tweak – GM used as lab tool

In some cases, a crop is developed using GM as a lab research tool, but no GM genes are added. Nevertheless, such crops have been claimed to be GM successes. An example is flood-tolerant rice, which the UK government's former chief scientist, Sir David King, has wrongly claimed as a triumph of genetic engineering.[56,57]

In fact, the two best-known flood-tolerant rice varieties – one of which was almost certainly the one that King referred to – are not GM at all. One variety was developed by a research team led by GM proponent Pamela Ronald.[58] Ronald's team developed the rice through marker assisted selection (MAS).[58,59] They used genetic engineering as a laboratory research tool to identify the desired genes, but the resulting rice is not genetically engineered.[60]

However, the wording on the website of UC Davis, where Ronald's laboratory is based, misleadingly implied that her rice was genetically engineered, saying, "Her laboratory has genetically engineered rice for resistance to diseases and flooding, which are serious problems of rice crops in Asia and Africa."[61]

Another flood-tolerant rice created with "Snorkel" genes has also been claimed as a genetic engineering success. But the rice, which adapts to flooding by growing longer stems that prevent the crop from drowning, was bred by conventional methods and is entirely non-GM.

Laboratory-based genetic modification and modern gene mapping methods were used to study a deepwater rice variety and identify the genes responsible for its flood tolerance trait. Three gene regions were identified, including one where the two "Snorkel" genes are located. MAS was used to guide the conventional breeding process by which all three flood tolerance gene regions were successfully combined in a commercial rice variety.[62]

Only conventional breeding and MAS could be used to generate the resulting flood-tolerant rice line. This is because it is beyond the ability of current genetic modification methods to transfer the genes and control switches for the flood-tolerance trait in a way that enables them to work properly.

Crop that has nothing to do with GM

In one high-profile case, a crop that had nothing to do with GM at all was claimed as a GM success. In a BBC radio interview, the UK government's former chief scientist, Sir David King, said that a big increase in grain yields in Africa was due to GM, when in fact it did not involve the use of GM technology.[63] Instead, the yield increase was due to a "push-pull" management system, an agroecological method of companion planting that aims to divert pests away from crop plants.[64] King later admitted to what he called an "honest mistake".[65]

King produced this example when under pressure to provide compelling reasons why GM crops are needed. But far from showing why we need to embrace GM, it shows the exact opposite – that we need to stop being distracted by GM and put funding and support behind non-GM solutions to urgent problems.

7.3.2. Non-GM breeding successes show no need for GM

The following are just a few examples of conventionally bred crops with the types of traits that GM proponents claim can only be achieved through genetic engineering. Many are already commercially available and making a difference in farmers' fields.

Drought-tolerant and climate-ready

- Maize varieties that yield well in drought conditions,[66] including some developed for farmers in Africa[67,68,69]
- Cassava that gives high yields in drought conditions and resists several diseases[10]
- Climate-adapted, high-yield sorghum varieties developed for farmers in Mali[70]
- Beans resistant to heat, drought, and disease[71,72]
- Pearl millet, sorghum, chickpea, pigeon pea and groundnut varieties that tolerate drought and high temperatures[73]
- Rice varieties bred to tolerate drought, flood, disease, and saline (salty) soils[74]
- Flood-tolerant rice varieties developed for Asia[75,76]
- Over 2,000 indigenous rice varieties that are adapted to environmental fluctuations, as well as varieties that resist pests and diseases, registered by Navdanya, a seed-keeping NGO based in India[77]
- Tomato varieties developed by Nepali farmers that tolerate extreme heat and resist disease.[78]

Salt-tolerant

- Rice varieties that tolerate saline soils and other problems[74]
- Durum wheat that yields 25% more in saline soils than a commonly used variety[79,80]
- Indigenous crop varieties from India that tolerate saline soils, stored by the Indian seed-keeping NGO, Navdanya. Navdanya reported that it gave some of these seeds to farmers in the wake of the 2004 tsunami, enabling them to continue farming in salt-saturated soils in spite of scientists' warnings that they would have to abandon the land temporarily.[81]
- High-yield, pest-resistant, and disease-resistant
- High-yield, multi-disease-resistant beans for farmers in Central and East Africa[82]
- High-yield, disease-resistant cassava for Africa[83]
- Australian high-yield maize varieties targeted at non-GM Asian markets[84]
- Maize that resists the Striga parasitic weed pest and tolerates drought, for African farmers[69]
- Maize that resists the grain borer pest[85]
- "Green Super-Rice" bred for high yield and disease resistance[74]
- High-yield soybeans that resist the cyst nematode pest[86]
- Aphid-resistant soybeans[87]
- High-yield tomato with sweeter fruit[88]
- High-yield, pest-resistant chickpeas[89]
- Sweet potato that is highly resistant to nematodes and moderately resistant to insect pests and Fusarium wilt, a fungal disease[90]
- High-yield, high-nutrition, and pest-resistant "superwheat"[91]
- Habanero peppers with resistance to root-knot nematodes.[92]
- Potatoes that resist late blight and other diseases[93,94,95,96]
- Potatoes that resist golden nematode and common scab – and appeal to food manufacturers due to good chipping and storage qualities[97]
- Potato that resists root-knot nematodes[98]
- Papayas that resist ringspot virus[99] – in spite of numerous claims from the GM lobby that only GM was able to produce a resistant papaya. Interestingly, there even seems to be doubt about the frequent claim that the GM virus-resistant papaya saved Hawaii's papaya industry. The GM papaya has dominated Hawaiian papaya production since the late 1990s, but Hawaii's Department of Agriculture reportedly said that the annual yield of papayas in 2009 was lower than when the ringspot virus was at its peak.[100]

An article in the Hawaii press said that GM has not saved Hawaii's papaya industry, which has been in decline since 2002. The article cites as a possible reason the market rejection that has plagued GM papayas from the beginning.[101]

Nutritionally fortified and health-promoting

- Soybeans containing high levels of oleic acid, reducing the need for hydrogenation, a process that leads to the formation of unhealthy trans fats[102]
- Beta-carotene-enriched orange maize, aimed at poor people suffering from vitamin A deficiency[103,104]
- Millet rich in iron, wheat abundant in zinc, and beta-carotene-enriched cassava[105]
- Iron-fortified maize, which has been shown in a study to decrease anaemia in children[106,107]
- Purple potatoes containing high levels of the cancer-fighting antioxidants, anthocyanins[108,109]
- A tomato containing high levels of the antioxidant, lycopene, which has been found in studies to have the potential to combat heart attacks, stroke, and cancer.[110]
- Low-allergy peanuts.[111] In a separate development, a process has been discovered to render ordinary peanuts allergen-free.[112]

7.3.3. Conventional breeding is quicker and cheaper than GM

"The overall cost to bring a new biotech trait to the market between 2008 and 2012 is on average $136 m[illion]."

– Phillips McDougall, "The cost and time involved in the discovery, development and authorisation of a new plant biotechnology derived trait: A consultancy study for Crop Life International"[113]

"Genetic engineering might be worth the extra cost if classical breeding were unable to impart such desirable traits as drought-, flood- and pest-resistance, and fertilizer efficiency. But in case after case, classical breeding is delivering the goods."

– Margaret Mellon and Doug Gurian-Sherman[51]

An industry consultancy study put the cost of developing a GM trait at $136 million.[113] Even Monsanto has admitted that non-GM plant breeding is quicker and "significantly cheaper" than GM. Monsanto said it takes ten years to develop a GM seed, in contrast with a conventionally bred variety, which takes only 5–8 years.[114] The plant breeder Major M. Goodman of North Carolina State University said the cost of developing a GM trait was fifty times as much as the cost of developing an equivalent conventionally bred plant variety. Goodman called the cost of GM breeding a "formidable barrier" to its expansion.[50]

Time and cost are vital considerations for the Global South, where the need for crop varieties adapted to local conditions is urgent, yet farmers cannot afford expensive seeds and inputs.

Conclusion to Section 7

GM crops are promoted as a way of solving world hunger. But this argument does not stand up to analysis, since there are no GM crops with a higher intrinsic yield or that cope better with challenging climate conditions than non-GM varieties.

Most GM crops are engineered to tolerate herbicides or to express a pesticide. They mostly go into biofuels, animal feed, and processed food – all products for affluent countries that have nothing to do with the food needs of the poor and hungry.

Hunger is in any case not caused by a lack of food in the world. It is a problem of distribution and poverty. Poor people have no money to buy food, and increasingly, no land on which to grow it.

A few GM crops have been developed to help poor farmers in Africa. But they have had disastrous results, leaving the farmers who adopted them worse off than before. In contrast, conventional breeding programs have developed non-GM crops far more cheaply and successfully.

Breeding improved crop varieties is part of the answer to food security – the other part is how crops are grown and land is managed. The

IAASTD report, commissioned by the World Bank and United Nations and authored by over 400 international experts and scientists, concluded that the key to food security lay in agroecological farming methods. The report did not endorse GM as a solution, noting that yields were "variable".

Other studies confirm that agroecological farming has resulted in significant yield and income benefits to farmers in the Global South, while preserving soil for future generations.

The expense of GM seeds and the chemical inputs on which they often rely make them irrelevant to solving the problem of hunger. GM seeds are patented and owned by multinational corporations and farmers are forbidden from saving seed to replant, shifting control of the food supply from farmers to corporations. While non-GM seed is also increasingly patented, the GM process lends itself more easily to patenting than conventional breeding.

Finally, GM is simply not needed to feed the world. Conventional plant breeding has successfully delivered crops that are high-yielding, disease- and pest-resistant, tolerant of drought and other climatic extremes, and nutritionally enhanced – at a fraction of the cost of GM.

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CONCLUSION

Genetically modified (GM) crops are promoted on the basis of far-reaching claims from the industry and its supporters, such as:

- Humans have been genetically modifying crops for centuries and genetic engineering is no different
- GM crops are safe for human and animal health and the environment
- GM crops increase yields and reduce pesticide use
- GM will produce supercrops that tolerate drought, resist pests and disease, and provide improved nutritional value
- GM crops are "an important tool in the toolbox" to feed the world.

However, based on the evidence presented in this report, these claims are misleading. The GM process is completely different from natural breeding and entails different risks. The GM transgene insertion and associated tissue culture processes are imprecise and highly mutagenic, causing unpredictable changes in the DNA, proteins, and biochemical composition of the resulting GM crop that can lead to unexpected toxic or allergenic effects and nutritional disturbances.

There is no scientific consensus that GM crops are safe, especially when the views of the scientific community independent of the GM crop development industry are taken into account. Toxicological studies in laboratory animals and livestock have revealed unexpected harmful effects from a diet containing GM crops, including some that are already in the human food and feed supply. Among the most marked effects are disturbances in liver and kidney function.

Many of these studies, including some conducted by the GM crop industry and others commissioned by the EU, have been incorrectly claimed by GM proponents to show that GM crops are safe when in fact, they show harmful effects. In some cases, advocates of GM crops have admitted that statistically significant differences were found between the GM and non-GM feeds under test but have dismissed them as "not biologically relevant/significant". However, these terms have not been defined and are scientifically meaningless.

Most animal feeding studies on GM crops have been relatively short – 30–90 days in length (technically called medium-term studies). What is needed are long-term and multi-generational studies to see if the worrying signs of toxicity observed in medium-term investigations develop into serious disease. Long-term studies of this type are not required for GM crops by government regulators anywhere in the world.

This and other inadequacies of the regulatory regime for GM crops and foods mean that it is too weak to protect consumers from the potential hazards posed by the technology. Regulation is weakest in the US and is inadequate in most regions of the world, including Europe.

GM crops have not delivered on their promises and, based on current evidence, it seems unlikely that they will provide sustainable solutions to the problems that face humanity, such as hunger and climate change.

Claims that GM technology will help feed the world are not credible in the light of the fact that GM technology has not increased the intrinsic yield of crops. While yields for major crops have increased in recent decades, this has been as a result of conventional breeding successes, not due to GM.

Also, the majority of GM crops are commodity crops grown on a large scale for affluent countries, such as soy and maize. A few GM crops have been developed for small-scale farmers in Africa, such as a sweet potato and cassava varieties that were intended to be virus-resistant, but these have failed miserably. In contrast, projects using conventional breeding have succeeded at a fraction of the cost of the GM projects.

GM crops have not decreased pesticide use, but have increased it. In particular, the widespread adoption of GM Roundup Ready crops has led to over-reliance on Roundup herbicide, leading to the spread of resistant weeds. This in turn has required farmers to spray more Roundup and

mixtures of chemicals in an attempt to control weeds.

Roundup is not safe or benign. It has been found to cause malformations in laboratory animals, to be toxic to human cells at very low doses, and to cause DNA damage in humans and animals. Epidemiological studies have found an association between Roundup exposure and cancer, premature births and miscarriages, and impaired neurological development in humans. In addition, Roundup applications can cause increases in plant diseases, including infection with Fusarium, a fungus that negatively impacts yields as well as producing toxins that can enter the food chain and affect the health of humans and livestock.

As Roundup fails under the onslaught of resistant weeds, the GM industry is developing multi-herbicide-tolerant crops that withstand being sprayed with potentially even more toxic herbicides, such as 2,4-D. These crops will lead to an immediate escalation in the use of these herbicides.

It is often claimed that GM Bt insecticidal crops reduce the need for chemical insecticide sprays. But these reductions, when they occur, are often temporary. Resistance has developed among target pests and even when control of the target pest has been successful, secondary pests have moved into the ecological niche. These developments demonstrate that GM Bt technology is not sustainable. In addition, Bt crops are themselves insecticide-containing plants, so even when they work as intended, they do not eliminate or reduce insecticides but simply change the way in which insecticides are used.

Advocates often claim that GM Bt crops are safe because Bt toxin has been safely used for decades as a spray to kill pests by chemical and organic farmers. But the Bt toxin expressed in GM plants is structurally very different from natural Bt used as a spray. The Bt toxin in GM plants is not always fully broken down in digestion and has been found to have toxic effects on laboratory animals and non-target organisms fed on such crops.

GM proponents have long promised climate-ready and drought-tolerant crops, but conventional breeding has been far more successful than GM technology in producing such crops. This is unsurprising, as these traits are genetically complex and cannot be produced by manipulating one or two genes.

GM herbicide-tolerant crops are often claimed to be climate-friendly because they are grown using the no-till farming method, which uses herbicides instead of ploughing to control weeds. No-till farming with GM crops is said to store carbon more effectively in the soil than ploughing, which releases carbon into the atmosphere as carbon dioxide. However, studies show that no-till fields do not store carbon more effectively than ploughed fields when deeper levels of soil are measured, throwing into question claims that no-till with GM crops offers a solution to climate change. In addition, the adoption of no-till with GM herbicide-tolerant crops has been found to increase the negative environmental impact of soy cultivation, because of the herbicides used.

Based on the evidence presented in this report, it is clear that GM technology has failed to deliver on its promises. GM technology is fundamentally unsound and poses scientifically proven risks to human and animal health, as well as the environment. The claims made for the benefits of GM crops are highly exaggerated and GM crop technology has been shown to be unsustainable.

It is not necessary to accept the risks posed by GM crops when conventional breeding – sometimes assisted by safe biotechnologies such as marker assisted selection – continues to successfully produce crops that are high-yielding, drought-tolerant, climate-ready, pest- and disease-resistant, and nutritious. Conventional breeding, the existing crop varieties developed by farmers worldwide, and agroecological farming methods, are proven effective methods of meeting our current and future food needs.

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Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada

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ABSTRACT

Pesticides associated to genetically modified foods (PAGMF), are engineered to tolerate herbicides such as glyphosate (GLYP) and gluphosinate (GLUF) or insecticides such as the bacterial toxin bacillus thuringiensis (Bt). The aim of this study was to evaluate the correlation between maternal and fetal exposure, and to determine exposure levels of GLYP and its metabolite aminomethyl phosphoric acid (AMPA), GLUF and its metabolite 3-methylphosphinicopropionic acid (3-MPPA) and Cry1Ab protein (a Bt toxin) in Eastern Townships of Quebec, Canada. Blood of thirty pregnant women (PW) and thirty-nine nonpregnant women (NPW) were studied. Serum GLYP and GLUF were detected in NPW and not detected in PW. Serum 3-MPPA and CryAb1 toxin were detected in PW, their fetuses and NPW. This is the first study to reveal the presence of circulating PAGMF in women with and without pregnancy, paving the way for a new field in reproductive toxicology including nutrition and utero-placental toxicities.

1. Introduction

An optimal exchange across the maternal-fetal unit (MFU) is necessary for a successful pregnancy. The placenta plays a major role in the embryo's nutrition and growth, in the regulation of the endocrine functions and in drug biotransformation [1–3]. Exchange involves not only physiological constituents, but also substances that represent a pathological risk for the fetus such as xenobiotics that include drugs, food additives, pesticides, and environmental pollutants [4]. The understanding of what xenobiotics do to the MFU and what the MFU does to the xenobiotics should provide the basis for the use of placenta as a tool to investigate and predict some aspects of developmental toxicity [4]. Moreover, pathological conditions in the placenta are important causes of intrauterine or perinatal death, congenital anomalies, intrauterine growth retardation, maternal death, and a great deal of morbidity for both, mother and child [5].

Genetically modified plants (GMP) were first approved for commercialization in Canada in 1996 then become distributed worldwide. Global areas of these GMP increased from 1.7 million hectares in 1996 to 134 million hectares in 2009, a 80-fold increase [6]. This growth rate makes GMP the fastest adopted crop technology [6]. GMP are plants in which genetic material has been altered in a way that does not occur naturally. Genetic engineering allows gene transfer (transgenesis) from an organism into another in order to confer them new traits. Combining GMP with pesticides-associated GM foods (PAGMF) allows the protection of desirable crops and the elimination of unwanted plants by reducing the competition for nutrients or by providing insect resistance. There is a debate on the direct threat of genes used in the preparation of these new foods on human health, as they are not detectable in the body, but the real danger may come from PAGMF [6–10]. Among the innumerable PAGMF, two categories are largely used in our agriculture since their introduction in 1996: (1) residues derived from herbicide-tolerant GM crops such as glyphosate (GLYP) and its metabolite aminomethyl phosphoric acid (AMPA) [11], and gluphosinate ammonium (GLUF) and its metabolite 3-methylphosphinicopropionic acid (MPPA) [12]; and (2) residues derived from insect-resistant GM crops such as Cry1Ab protein [13,14].

Among herbicide-tolerant GM crops, the first to be grown commercially were soybeans which were modified to tolerate glyphosate [11]. Glyphosate [*N*-(phosphonomethyl) glycine] is a nonselective, post-emergence herbicide used for the control of a

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wide range of weeds [15]. It can be used on non-crop land as well as in a great variety of crops. GLYP is the active ingredient in the commercial herbicide Roundup®. Glyphosate is an acid, but usually used in a salt form, most commonly the isopropylamine salt. The target of glyphosate is 5-enolpyruvoylshikimate 3-phosphate synthase (EPSPS), an enzyme in the shikimate pathway that is required for the synthesis of many aromatic plant metabolites, including some amino acids. The gene that confers tolerance of the herbicide is from the soil bacterium *Agrobacterium tumefaciens* and makes an EPSPS that is not affected by glyphosate. Few studies have examined the kinetics of absorption, distribution, metabolism and elimination (ADME) of glyphosate in humans [15,16]. Curwin et al. [17] reported detection of urinary GLYP concentrations among children, mothers and fathers living in farm and non farm households in Iowa. The ranges of detection were 0.062–5.0 ng/ml and 0.10–11 ng/ml for non farm and farm mothers, respectively. There was no significant difference between farm and non farm mothers and no positive association between the mothers' urinary glyphosate levels and glyphosate dust concentrations. These findings suggest that other sources of exposure such as diet may be involved.

Gluphosinate (or glufosinate) [ammonium dl-homoalanin-4-(methyl) phosphinate] is a broad-spectrum, contact herbicide. Its major metabolite is 3-methylphosphinicopropionic acid (MPPA), with which it has similar biological and toxicological effects [18]. GLUF is used to control a wide range of weeds after the crop emerges or for total vegetation control on land not used for cultivation. Gluphosinate herbicides are also used to desiccate (dry out) crops before harvest. It is a phosphorus-containing amino acid. It inhibits the activity of an enzyme, glutamine synthetase, which is necessary for the production of the amino acid glutamine and for ammonia detoxification [12]. The application of GLUF leads to reduced glutamine and increased ammonia levels in the plant's tissues. This causes photosynthesis to stop and the plant dies within a few days. GLUF also inhibits the same enzyme in animals [19]. The gene used to make plants resistant to gluphosinate comes from the bacterium *Streptomyces hygroscopicus* and encodes an enzyme called phosphinothricine acetyl transferase (PAT). This enzyme detoxifies GLUF. Crop varieties carrying this trait include varieties of oilseed rape, maize, soybeans, sugar beet, fodder beet, cotton and rice. As for GLYP, its kinetics of absorption, distribution, metabolism and elimination (ADME) is not well studied in humans, except few poisoned-case studies [16,20,21]. Hirose et al. reported the case of a 65-year-old male who ingested BASTA, which contains 20% (w/v) of GLUF ammonium, about 300 ml, more than the estimated human toxic dose [20]. The authors studied the serial change of serum GLUF concentration every 3–6 h and assessed the urinary excretion of GLUF every 24 h. The absorbed amount of GLUF was estimated from the cumulative urinary excretion. The changes in serum GLUF concentration exhibited $T_{1/2\alpha}$ of 1.84 and $T_{1/2\alpha}$ of 9.59 h. The apparent distribution volume at b-phase and the total body clearance were 1.44 l/kg and 86.6 ml/min, respectively. Renal clearance was estimated to be 77.9 ml/min.

The Cry1Ab toxin is an insecticidal protein produced by the naturally occurring soil bacterium *Bacillus thuringiensis* [22,23]. The gene (truncated *cry1Ab* gene) encoding this insecticidal protein was genetically transformed into maize genome to produce a transgenic insect-resistant plant (Bt-maize; MON810) and, thereby, provide specific protection against Lepidoptera infestation [13,14]. For more than 10 years, GM crops have been commercialized and approved as an animal feed in several countries worldwide. The Cry toxins (protoxins) produced by GM crops are solubilized and activated to Cry toxins by gut proteases of susceptible insect larvae. Activated toxin binds to specific receptors localized in the midgut epithelial cells [24,25], invading the cell membrane and forming cation-selective ion channels that lead to the disruption of the epithelial barrier and larval death by osmotic cell lysis [26–28].

Since the basis of better health is prevention, one would hope that we can develop procedures to avoid environmentally induced disease in susceptible population such as pregnant women and their fetuses. The fetus is considered to be highly susceptible to the adverse effects of xenobiotics. This is because environmental agents could disrupt the biological events that are required to ensure normal growth and development [29,30]. PAGMF are among the xenobiotics that have recently emerged and extensively entered the human food chain [9], paving the way for a new field of multidisciplinary research, combining human reproduction, toxicology and nutrition, but not as yet explored. Generated data will help regulatory agencies responsible for the protection of human health to make better decisions. Thus, the aim of this study was to investigate whether pregnant women are exposed to PAGMF and whether these toxicants cross the placenta to reach the fetus.

2. Materials and methods

2.1. Chemicals and reagents

For the analytical support (Section 2.3), GLYP, AMPA, GLUF, APPA and *N*-methyl-*N*-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) + 1% tert-buryldimethylchlorosilane (TBDMCS) were purchased from Sigma (St. Louis, MO, USA). 3-MPPA was purchased from Wako Chemicals USA (Richmond, VA, USA) and Sep-Pak Plus PS-2 cartridges, from Waters Corporation (Milford, MA, USA). All other chemicals and reagents were of analytical grade (Sigma, MO, USA). The serum samples for validation were collected from volunteers.

2.2. Study subjects and blood sampling

At the Centre Hospitalier Universitaire de Sherbrooke (CHUS), we formed two groups of subjects: (1) a group of healthy pregnant women ($n = 30$), recruited at delivery; and (2) a group of healthy fertile nonpregnant women ($n = 39$), recruited during their tubal ligation of sterilization. As shown in Table 1 of clinical characteristics of subjects, eligible groups were matched for age and body mass index (BMI). Participants were not known for cigarette or illicit drug use or for medical condition (i.e. diabetes, hypertension or metabolic disease). Pregnant women had vaginal delivery and did not have any adverse perinatal outcomes. All neonates were of appropriate size for gestational age (3423 ± 375 g).

Blood sampling was done before delivery for pregnant women or at tubal ligation for nonpregnant women and was most commonly obtained from the median cubital vein, on the anterior forearm. Umbilical cord blood sampling was done after birth using the syringe method. Since labor time can take several hours, the time between taking the last meal and blood sampling is often a matter of hours. Blood samples were collected in BD Vacutainer 10 ml glass serum tubes (Franklin Lakes, NJ, USA). To obtain serum, whole blood was centrifuged at 2000 rpm for 15 min within 1 h of collection. For maternal samples, about 10 ml of blood was collected, resulting in 5–6.5 ml of serum. For cord blood samples, about 10 ml of blood was also collected by syringe, giving 3–4.5 ml of serum. Serum was stored at −20 °C until assayed for PAGMF levels.

Subjects were pregnant and non-pregnant women living in Sherbrooke, an urban area of Eastern Townships of Quebec, Canada. No subject had worked or lived with a spouse working in contact with pesticides. The diet taken is typical of a middle

Table 1
Characteristics of subjects.

	Pregnant women ($n = 30$)	Nonpregnant women ($n = 39$)	*P* value[a]
Age (year, mean ± SD)	32.4 ± 4.2	33.9 ± 4.0	NS
BMI (kg/m^2, mean ± SD)	24.9 ± 3.1	24.8 ± 3.4	NS
Gestational age (week, mean ± SD)	38.3 ± 2.5	N/A	N/A
Birth weight (g, mean ± SD)	3364 ± 335	N/A	N/A

BMI, body mass index; N/A, not applicable; data are expressed as mean ± SD; NS, not significant.

[a] *P* values were determined by Mann–Whitney test.

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class population of Western industrialized countries. A food market-basket, representative for the general Sherbrooke population, contains various meats, margarine, canola oil, rice, corn, grain, peanuts, potatoes, fruits and vegetables, eggs, poultry, meat and fish. Beverages include milk, juice, tea, coffee, bottled water, soft drinks and beer. Most of these foods come mainly from the province of Quebec, then the rest of Canada and the United States of America. Our study did not quantify the exact levels of PAGMF in a market-basket study. However, given the widespread use of GM foods in the local daily diet (soybeans, corn, potatoes, . . .), it is conceivable that the majority of the population is exposed through their daily diet [31,32].

The study was approved by the CHUS Ethics Human Research Committee on Clinical Research. All participants gave written consent.

2.3. Herbicide and metabolite determination

Levels of GLYP, AMPA, GLUF and 3-MPPA were measured using gas chromatography–mass spectrometry (GC–MS).

2.3.1. Calibration curve

According to a method described by Motojyuku et al. [16], GLYP, AMPA, GLUF and 3-MPPA (1 mg/ml) were prepared in 10% methanol, which is used for all standards dilutions. These solutions were further diluted to concentrations of 100 and 10 μg/ml and stored for a maximum of 3 months at 4 °C. A 1 μg/ml solution from previous components was made prior herbicide extraction. These solutions were used as calibrators. A stock solution of DL-2-amino-3-phosphonopropionic acid (APPA) (1 mg/ml) was prepared and used as an internal standard (IS). The IS stock solution was further diluted to a concentration of 100 μg/ml. Blank serum samples (0.2 ml) were spiked with 5 μl of IS (100 μg/ml), 5 μl of each calibrator solution (100 μg/ml), or 10, 5 μl of 10 μg/ml solution, or 10, 5 μl of 1 μg/ml solution, resulting in calibration samples containing 0.5 μg of IS (2.5 μg/ml), with 0.5 μg (2.5 μg/ml), 0.1 μg (0.5 μg/ml), 0.05 μg (0.25 μg/ml), 0.01 μg (0.05 μg/ml) 0.005 μg (0.025 μg/ml) of each compound (i.e. GLYP, AMPA, GLUF and 3-MPPA). Concerning extraction development, spiked serum with 5 μg/ml of each compound was used as control sample.

2.3.2. Extraction procedure

The calibration curves and serum samples were extracted by employing a solid phase extraction (SPE) technique, modified from manufacturer's recommendations and from Motojyuku et al. [16]. Spiked serum (0.2 ml), prepared as described above, and acetonitrile (0.2 ml) were added to centrifuge tubes. The tubes were then vortexed (15 s) and centrifuged (5 min, 1600 × *g*). The samples were purified by SPE using 100 mg Sep-Pak Plus PS-2 cartridges, which were conditioned by washing with 4 ml of acetonitrile followed by 4 ml of distilled water. The samples were loaded onto the SPE cartridges, dried (3 min, 5 psi) and eluted with 2 ml of acetonitrile. The solvent was evaporated to dryness under nitrogen. The samples were reconstituted in 50 μl each of MTBSTFA with 1% TBDMCS and acetonitrile. The mixture was vortexed for 30 s every 10 min, 6 times. Samples of solution containing the derivatives were used directly for GC–MS (Agilent Technologies 6890N GC and 5973 Invert MS).

2.3.3. GC–MS analysis

Chromatographic conditions for these analyses were as followed: a 30 m × 0.25 mm Zebron ZB-5MS fused-silica capillary column with a film thickness of 0.25 μm from Phenomenex (Torrance, CA, USA) was used. Helium was used as a carrier gas at 1.1 ml/min. A 2 μl extract was injected in a split mode at an injection temperature of 250 °C. The oven temperature was programmed to increase from an initial temperature of 100 °C (held for 3 min) to 300 °C (held for 5 min) at 5 °C/min. The temperatures of the quadrupode, ion source and mass-selective detector interface were respectively 150, 230 and 280 °C. The MS was operated in the selected-ion monitoring (SIM) mode. The following ions were monitored (with quantitative ions in parentheses): GLYP (454), 352; AMPA (396), 367; GLUF (466); 3-MPPA (323); IS (568), 466.

The limit of detection (LOD) is defined as a signal of three times the noise. For 0.2 ml serum samples, LOD was 15, 10, 10 and 5 ng/ml for GLYP, GLUF, AMPA and 3-MPPA, respectively.

2.4. Cry1Ab protein determination

Cry1Ab protein levels were determined in blood using a commercially available double antibody sandwich (DAS) enzyme-linked immunosorbent assay (Agdia, Elkhart, IN, USA), following manufacturer's instructions. A standard curve was prepared by successive dilutions (0.1–10 ng/ml) of purified Cry1Ab protein (Fitzgerald Industries International, North Acton, MA, USA) in PBST buffer. The mean absorbance (650 nm) was calculated and used to determine samples concentration. Positive and negative controls were prepared with the kit Cry1Ab positive control solution, diluted 1/2 in serum.

2.5. Statistical analysis

PAGMP exposure was expressed as number, range and mean ± SD for each group. Characteristics of cases and controls and PAGMP exposure were compared using the Mann–Whitney *U*-test for continuous data and by Fisher's exact test for categorical data. Wilcoxon matched pairs test compared two dependent groups.

Table 2
Concentrations of GLYP, AMPA, GLUF, 3-MPPA and Cry1Ab protein in maternal and fetal cord serum.

	Maternal ($n = 30$)	Fetal cord ($n = 30$)	*P* value[a]
GLYP			
Number of detection	nd	nd	nc
Range of detection (ng/ml)			
Mean ± SD			
AMPA			
Number of detection	nd	nd	nc
Range of detection (ng/ml)			
Mean ± SD (ng/ml)			
GLUF			
Number of detection	nd	nd	nc
Range of detection (ng/ml)			
Mean ± SD (ng/ml)			
3-MPPA			
Number of detection	30/30 (100%)	30/30 (100%)	$P < 0.001$
Range of detection (ng/ml)	21.9–417	8.76–193	
Mean ± SD (ng/ml)	120 ± 87.0	57.2 ± 45.6	
Cry1Ab			
Number of detection	28/30 (93%)	24/30 (80%)	$P = 0.002$
Range of detection (ng/ml)	nd–1.50	nd–0.14	
Mean ± SD (ng/ml)	0.19 ± 0.30	0.04 ± 0.04	

GLYP, glyphosate; AMPA, aminomethyl phosphoric acid; GLUF, gluphosinate ammonium; 3-MPPA, 3-methylphosphinicopropionic acid; Cry1Ab, protein from bacillus thuringiensis; nd, not detectable; nc, not calculable because not detectable. Data are expressed as number (*n*, %) of detection, range and mean ± SD (ng/ml).

[a] *P* values were determined by Wilcoxon matched pairs test.

Other statistical analyses were performed using Spearman correlations. Analyses were realized with the software SPSS version 17.0. A value of $P < 0.05$ was considered as significant for every statistical analysis.

3. Results

As shown in Table 1, pregnant women and nonpregnant women were similar in terms of age and body mass index. Pregnant women had normal deliveries and birth-weight infants (Table 1).

GLYP and GLUF were non-detectable (nd) in maternal and fetal serum, but detected in nonpregnant women (Table 2, Fig. 1). GLYP was [2/39 (5%), range (nd–93.6 ng/ml) and mean ± SD (73.6 ± 28.2 ng/ml)] and GLUF was [7/39 (18%), range (nd–53.6 ng/ml) and mean ± SD (28.7 ± 15.7 ng/ml). AMPA was not detected in maternal, fetal and nonpregnant women samples. The metabolite 3-MPPA was detected in maternal serum [30/30 (100%), range (21.9–417 ng/ml) and mean ± SD (120 ± 87.0 ng/ml), in fetal cord serum [30/30 (100%), range (8.76–193 ng/ml) and mean ± SD (57.2 ± 45.6 ng/ml) and in nonpregnant women serum [26/39 (67%), range (nd–337 ng/ml) and mean ± SD (84.1 ± 70.3 ng/ml)]. A significant difference in 3-MPPA levels was evident between maternal and fetal serum ($P < 0.001$, Table 2, Fig. 1), but not between maternal and nonpregnant women serum ($P = 0.075$, Table 3, Fig. 1).

Serum insecticide Cry1Ab toxin was detected in: (1) pregnant women [28/30 (93%), range (nd–1.5 ng/ml) and mean ± SD (0.19 ± 0.30 ng/ml)]; (2) nonpregnant women [27/39 (69%), range (nd–2.28 ng/ml) and mean ± SD (0.13 ± 0.37 ng/ml)]; and (3) fetal cord [24/30 (80%), range (nd–0.14 ng/ml) and mean ± SD (0.04 ± 0.04 ng/ml)]. A significant difference in Cry1Ab levels was evident between pregnant and nonpregnant women's serum ($P = 0.006$, Table 3, Fig. 2) and between maternal and fetal serum ($P = 0.002$, Table 2, Fig. 2).

We also investigated a possible correlation between the different contaminants in the same woman. In pregnant women, GLYP, its metabolite AMPA and GLUF were undetectable in maternal blood and therefore impossible to establish a correlation between them. In nonpregnant women, GLYP was detected in 5% of the subjects, its metabolite AMPA was not detected and GLUF was detected in 18%, thus no significant correlation emerged from these contam-

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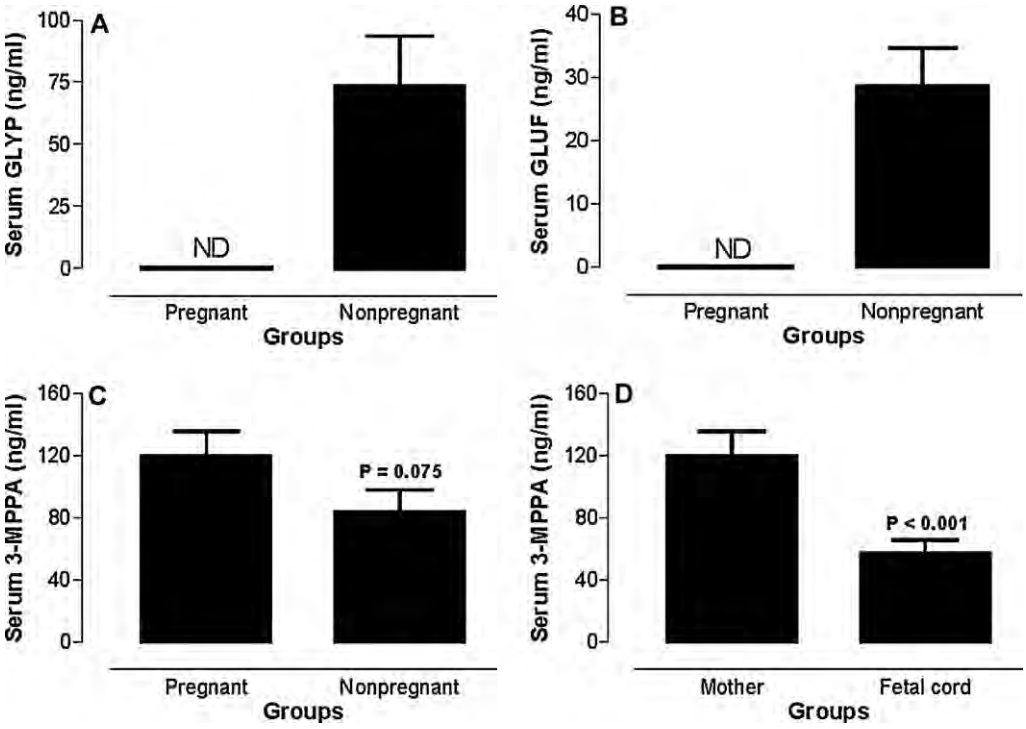

Fig. 1. Circulating concentrations of Glyphosate (GLYP: A), Gluphosinate (GLUF: B) and 3-methylphosphinicopropionic acid (3-MPPA: C and D) in pregnant and nonpregnant women (A–C) and in maternal and fetal cord blood (D). Blood sampling was performed from thirty pregnant women and thirty-nine nonpregnant women. Chemicals were assessed using GC–MS. *P* values were determined by Mann–Whitney test in the comparison of pregnant women to nonpregnant women (A–C). *P* values were determined by Wilcoxon matched pairs test in the comparison of maternal to fetal samples (D). A *P* value of 0.05 was considered as significant.

Table 3
Concentrations of GLYP, AMPA, GLUF, 3-MPPA and Cry1Ab protein in serum of pregnant and nonpregnant women.

	Pregnant women ($n = 30$)	Nonpregnant women ($n = 39$)	*P* value[a]
GLYP			
Number of detection	nd	2/39 (5%)	nc
Range of detection (ng/ml)		nd–93.6	
Mean ± SD		73.6 ± 28.2	
AMPA			
Number of detection	nd	nd	nc
Range of detection (ng/ml)			
Mean ± SD (ng/ml)			
GLUF			
Number of detection	nd	7/39 (18%)	nc
Range of detection (ng/ml)		nd–53.6	
Mean ± SD (ng/ml)		28.7 ± 15.7	
3-MPPA			
Number of detection	30/30 (100%)	26/39 (67%)	$P = 0.075$
Range of detection (ng/ml)	21.9–417	nd–337	
Mean ± SD (ng/ml)	120 ± 87.0	84.1 ± 70.3	
Cry1Ab			
Number of detection	28/30 (93%)	27/39 (69%)	$P = 0.006$
Range of detection (ng/ml)	nd–1.50	nd–2.28	
Mean ± SD (ng/ml)	0.19 ± 0.30	0.13 ± 0.37	

GLYP, glyphosate; AMPA, aminomethyl phosphoric acid; GLUF, gluphosinate ammonium; 3-MPPA, 3-methylphosphinicopropionic acid; Cry1Ab, protein from bacillus thuringiensis; nd, not detectable; nc, not calculable because not detectable. Data are expressed as number (*n*, %) of detection, range and mean ± SD (ng/ml).

[a] *P* values were determined by Mann–Whitney test.

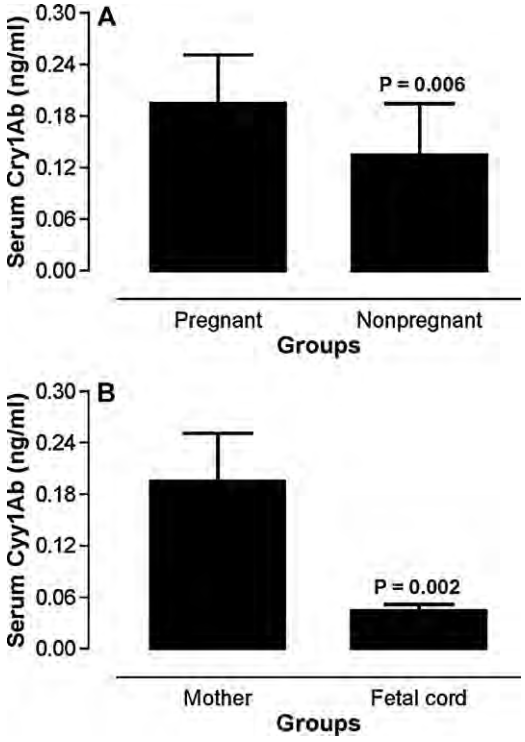

Fig. 2. Circulating concentrations of Cry1Ab toxin in pregnant and nonpregnant women (A), and maternal and fetal cord (B). Blood sampling was performed from thirty pregnant women and thirty-nine nonpregnant women. Levels of Cry1Ab toxin were assessed using an ELISA method. *P* values were determined by Mann–Whitney test in the comparison of pregnant women to nonpregnant women (A). *P* values were determined by Wilcoxon matched pairs test in the comparison of maternal to fetal samples (B). A *P* value of 0.05 was considered as significant.

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inants in the same subjects. Moreover, there was no correlation between 3-MPPA and Cry1Ab in the same women, both pregnant and not pregnant.

4. Discussion

Our results show that GLYP was not detected in maternal and fetal blood, but present in the blood of some nonpregnant women (5%), whereas its metabolite AMPA was not detected in all analyzed samples. This is may be explained by the absence of exposure, the efficiency of elimination or the limitation of the method of detection. Previous studies report that glyphosate and AMPA share similar toxicological profiles. Glyphosate toxicity has been shown to be involved in the induction of developmental retardation of fetal skeleton [33] and significant adverse effects on the reproductive system of male Wistar rats at puberty and during adulthood [34]. Also, glyphosate was harmful to human placental cells [35,36] and embryonic cells [36]. It is interesting to note that all of these animal and *in vitro* studies used very high concentrations of GLYP compared to the human levels found in our studies. In this regard, our results represent actual concentrations detected in humans and therefore they constitute a referential basis for future investigations in this field.

GLUF was detected in 18% of nonpregnant women's blood and not detected in maternal and fetal blood. As for GLYP, the non detection of GLUF may be explained by the absence of exposure, the efficiency of elimination or the limitation of the method of detection. Regarding the non-detection of certain chemicals in pregnant women compared with non pregnant women, it is assumed that the hemodilution caused by pregnancy may explain, at least in part, such non-detection. On the other hand, 3-MPPA (the metabolite of GLUF) was detected in 100% of maternal and umbilical cord blood samples, and in 67% of the nonpregnant women's blood samples. This highlights that this metabolite is more detectable than its precursor and seems to easily cross the placenta to reach the fetus. Garcia et al. [37] investigated the potential teratogenic effects of GLUF in humans found and increased risk of congenital malformations with exposure to GLUF. GLUF has also been shown in mouse embryos to cause growth retardation, increased death or hypoplasia [18]. As for GLYP, it is interesting to note that the GLUF concentrations used in these tests are very high (10 ug/ml) compared to the levels we found in this study (53.6 ng/ml). Hence, our data which provide the actual and precise concentrations of these toxicants, will help in the design of more relevant studies in the future.

On the other hand, Cry1Ab toxin was detected in 93% and 80% of maternal and fetal blood samples, respectively and in 69% of tested blood samples from nonpregnant women. There are no other studies for comparison with our results. However, trace amounts of the Cry1Ab toxin were detected in the gastrointestinal contents of livestock fed on GM corn [38–40], raising concerns about this toxin in insect-resistant GM crops; (1) that these toxins may not be effectively eliminated in humans and (2) there may be a high risk of exposure through consumption of contaminated meat.

5. Conclusions

To our knowledge, this is the first study to highlight the presence of pesticides-associated genetically modified foods in maternal, fetal and nonpregnant women's blood. 3-MPPA and Cry1Ab toxin are clearly detectable and appear to cross the placenta to the fetus. Given the potential toxicity of these environmental pollutants and the fragility of the fetus, more studies are needed, particularly those using the placental transfer approach [41]. Thus, our present results will provide baseline data for future studies exploring a new area of research relating to nutrition, toxicology and reproduction in women. Today, obstetric-gynecological disorders that are associated with environmental chemicals are not known. This may involve perinatal complications (i.e. abortion, prematurity, intrauterine growth restriction and preeclampsia) and reproductive disorders (i.e. infertility, endometriosis and gynecological cancer). Thus, knowing the actual PAGMF concentrations in humans constitutes a cornerstone in the advancement of research in this area.

Conflict of interest statement

The authors declare that they have no competing interests.

Acknowledgments

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THE ANATOMICAL RECORD 292:587–594 (2009)

The Impact of Dietary Organic and Transgenic Soy on the Reproductive System of Female Adult Rat

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ABSTRACT

The goal of this article was to compare the effects of a prolonged use of organic and transgenic soy on the lipid profile and ovary and uterus morphology. Wistar rats were fed three different diets from weaning until sacrifice at 15 months of age. The three diets were: casein-based diet control group (CG), organic soy-based diet group (OSG), or transgenic soy-based diet group (GMSG). There were no differences in food consumption or in the diet isoflavone components among the groups. Compared with the CG diet, both the OSG and GMSG diets were associated with significant reductions in body weight, serum triglycerides, and cholesterol ($P < 0.05$) (CG = 406 ± 23.1; 104.3 ± 13.2; 119.9 ± 7.3 GMSG = 368 ± 17.6; 60.3 ± 4.6; 83.3 ± 5.7 OSG = 389 ± 23.5; 72.3 ± 12.5; 95.5 ± 8.0, respectively). The volume density of endometrial glandular epithelium was greater in the GMSG group (29.5 ± 7.17, $P < 0.001$) when compared with the CG (18.5 ± 7.4) and OSG (20.3 ± 10.6) groups. The length density of endometrial glandular epithelium was shorter in both GMSG (567.6 ± 41.1) and OSG (514.8 ± 144.5) diets compared with the CG ($P < 0.05$) diet. GMSG also resulted in reduced follicle number and increased corpus luteum number compared to the OSG or CG diets ($P < 0.05$). In summary, both GMSG and OSG diets resulted in decreased body weight and lower serum triglyceride and cholesterol levels, and alterations in uterine and ovarian morphology were also observed. The prolonged use of soy-based diets and their relation to reproductive health warrants further investigation. Anat Rec, 292:587–594, 2009.

Key words: transgenic soy; endometrium; ovary; estradiol; cholesterol

Concerns have recently been raised regarding potential risks with soy protein formulae, in particular regarding their high phytoestrogenic isoflavone content. The main consumers for soy consumption include infants with severe lactose intolerance, glactosemia, dietary protein allergy, and infants of vegetarian parents (Turck, 2007). Soyfood has also been used to improve cardiovascular disease risk factors (Anthony et al., 1996; Bairey et al., 2006; Kohno et al., 2006) and to reduce risk, development, or incidence of breast cancer (Jin and Mac Donald, 2002; Liu et al., 2005).

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TABLE 1. Chemical composition of diets

	CG	GMSG	OSG
Water %	2.61 ± 0.51	2.72 ± 0.06	2.17 ± 0.01
Fat %	7.88 ± 0.12	8.53 ± 0.14	8.17 ± 0.11
Minerals %	1.67 ± 0.01	2.11 ± 0.08	2.14 ± 0.06
Protein %	10.95 ± 0.43	12.96 ± 1.18	11.05 ± 0.31
Fiber %	4.78 ± 0.75	4.88 ± 0.43	5.01 ± 0.36
Carbohydrate %	73.87	69.66	69.45
Calories/100 g	1,522.766 kJ	1,522.766 kJ	1,522.766 kJ

CG, control group; GMSG, genetically modified soy group; OSG, organic soy group. Results are present as mean ± standard deviation of three determinations.

Several studies have demonstrated a relationship between soy consumption and uterine and ovarian morphology and function. The majority report exposure to soy or any soy derivative during neonatal or adult life can cause abnormal estrous cycles, altered ovarian function, early reproductive senescence, subfertility/infertility, and uterotropic effects. These findings lead to questions about the safety of soy-based food consumption by women of reproductive age (Jefferson et al., 2002, 2005, 2006; Michael et al., 2006; Piotrowska et al., 2006; Rachon et al., 2007a,b).

Increased dietary soy consumption has lead to the development of transgenically produced soy to increase production and reduce associated cost (Rott et al., 2004). Transgenic soy is a genetically modified organism to which three foreign genes are added, one of them from a virus and the others from a bacterium found in soil. This modification provides the soy plant with resistance to glyphosate herbicides used to destroy weeds that compete with the crop.

On the other hand, organic soy is grown in an ecological manner, without chemical products that would contaminate or modify the product. Organically produced soy, however, results in a significant loss in productivity and increased cost (Magkos et al., 2003).

On the basis of the concern about the use of genetically modified food on health and the lack of data on the effects of transgenic soy on the reproductive female system, the goal of this article was to compare the effects of a prolonged use of organic and transgenic soy on the lipid profile in serum, and ovary and uterus morphology.

MATERIAL AND METHODS

Animals

The handling of the animals was approved by the Animal Care and Use Committee of State University of Rio de Janeiro, which based their analysis on the Guide for the Care and Use of Laboratory Animals (Bayne, 1996), and the study design was approved by the local Ethical Committee for the care and use of laboratory animals.

The biological assay was conducted on 24 female Wistar rats from the Laboratory of Experimental Nutrition (LABNE) of the Department of Nutrition and Dietetics, Nutrition College, Fluminense Federal University. The rats were divided into three groups of eight animals each, which received the experimental diets, as follows: control group (CG) fed a casein-based diet, organic soy group (OSG) fed an organic soy-based diet supplemented with 0.3 g cysteine, and a genetically modified soy group (GMSG) receiving a transgenic soy-based diet. As recommended by the American Institute of Nutrition-93, cystine was added to the OSG diet as a metionine precursor. However, because of concerns about genetic modification, we decided to not make an additional manipulation of the transgenic soy-based diet, so no cysteine was added to this diet.

During the studies, the rats were kept in polypropylene cages, in an environment with controlled temperature at 22°C and a 12-h light/dark period. Water and diets were offered ad libitum. Food consumption and animal weight were recorded daily.

To evaluate the prolonged use of soy by two generations, the animals used in this study were the offspring of parents (preceding generation) who also received the same diet throughout their lives. The animals were fed the above diets exclusively, from weaning until they were 15 months old. At the end of this period, the animals were euthanized under thiopental anesthesia (0.10 mL/100 g body weight), blood collection was made through cardiac puncture, and serum stored at −20°C to determine 17β-estradiol, cholesterol, and triglyceride serum levels. The left ovary and horn of the uterus were carefully removed, weighed, and fragmented according the Ortrip method (Mandarim-de-Lacerda, 2003). The material obtained was fixed in formalin (pH 7.2) and processed following the routine histological procedures for embedment in paraffin. Sections of 5 μm of thickness were stained by the hematoxylin and eosin for the analysis of the integrity of the specimens and exclusion of the samples that had artifacts.

Diets

Transgenic soy was supplied by Jasmine Integral Foods (Curitiba, PR, Brazil) and organic soy was supplied by Bunge Foods (Porto Alegre, RS, Brazil). The soybeans were processed as described in Soares et al. (2005) to minimize the antinutritional factors, and then the beans were used as the protein source for diet preparation. All diets were prepared in the LABNE according to the rules of the Committee on Laboratory Animal Diets, 1979, modified according to the recommendations of the American Institute of Nutrition-93 (Reeves et al., 1993) and the chemical composition are shown in Table 1. The ingredients of the diets were homogenized in an industrial mixer with boiling water. The mass obtained was transformed into tablets, which were dried in a ventilated oven at 60°C for 24 h, properly identified and stored refrigeration until the time for use.

The isoflavone content was determined as described by Klump et al. (2001). Briefly, samples of organic and transgenic soy were extracted at 65°C with methanol-

TABLE 2. Total and individual isoflavone components of diets (mg/g diet)

Groups	Total Isofl (mg/g)	Daidzein (mg/g)	Genistein (mg/g)	Daidzin (mg/g)	Glicitin (mg/g)	Genistin (mg/g)
GMSG	0.396 ± 0.03	0.032 ± 0.003	0.038 ± 0.003	0.063 ± 0.002	0.014 ± 0.002	0.249 ± 0.05
OSG	0.384 ± 0.04	0.030 ± 0.004	0.034 ± 0.002	0.067 ± 0.005	0.018 ± 0.001	0.235 ± 0.06

GMSG, genetically modified soy group; OSG, organic soy group. Data are reported as mean ± standard deviation of eight animals. The CG (control group) did not contain isoflavones.

TABLE 3. Body, ovary, and uterus weights, ovary and uterus relative weight, cholesterol, triglycerides, and estradiol serum levels

	CG Mean ± SD	GMSG Mean ± SD	OSG Mean ± SD
Body weight (g)	406 ± 23.1[a]	368 ± 17.6[b]	389 ± 23.5[a]
Ovary weight (g)	0.1 ± 0.03[a]	0.1 ± 0.02[a]	0.1 ± 0.02[a]
Ovary relative weight (mg tissue/g body weight)	0.02 ± 0.01[a]	0.03 ± 0.01[a]	0.02 ± 0.01[a]
Uterus weight (g)	0.5 ± 0.1[a]	0.5 ± 0.1[a]	0.4 ± 0.1[a]
Uterus relative weight (mg tissue/g body weight)	0.1 ± 0.03[a]	0.1 ± 0.03[a]	0.1 ± 0.04[a]
Cholesterol (mg/dL)	119.9 ± 7.3[a]	83.3 ± 5.7[b]	95.5 ± 8.0[b]
Triglycerides (mg/dL)	104.3 ± 13.2[a]	60.3 ± 4.6[b]	72.3 ± 12.5[b]
Estradiol (pg/dL)	149.3 ± 1.0[a]	94.7 ± 15.4[b]	102 ± 6.1[b]

CG, control group; GMSG, genetically modified soy group; OSG, organic soy group.
Values are given as mean ± standard deviation of eight animals. Different superscript letter in the same row means statistically significant differences.

water (80 + 20), saponified with dilute sodium hydroxide solution, and analyzed by reversed phase liquid chromatography, with UV detection at 260 nm. The data were analyzed for individual isoflavone components, subtotals of daidzin, daidzein, glycitin, genistin, and genistein (Table 2).

Stereological Parameters

Sections of 5 μm of thickness were stained with Gomoris' Trichrome (Bradbury and Rae, 1996). The M42 multipurpose test-system was used to quantify the endometrial compartment of the uterus (Mandarim-de-Lacerda, 2003). From each uterus, five different sections were selected from five fragments. Then, five random fields were evaluated from each section at 200× final magnification. Therefore, there were 25 test areas from each uterus. The stereological parameters analyzed were: (1) Volumetric density (Vv) of the glandular epithelium and (2) Length density (Lv) of the endometrial glands (Lv = 2 QA (mm/mm^3), where QA is the number of the glandular profiles in the test area).

Morphologic Classification of Follicles

Sections stained by hematoxylin and eosin (Bradbury and Rae, 1996) were taken at intervals of 50 μm to avoid the same follicle being counted twice. The 5 μm sections were digitized using a video camera coupled to a light microscope with 400× final magnification for primordial follicles and 100× for primary, preantral, antral, and Graafian follicles, and corpus luteum. Follicle types in ovarian cross-sections were defined as follows. Primary follicles comprised an oocyte surrounded by a single layer of cuboidal granulosa cells. Preantral follicles comprised an oocyte surrounded by two or more layers of granulosa cells with no antrum. Antral follicles were distinguished by the presence of an antrum within the granulosa cell layers enclosing the oocyte (Cheng et al., 2002).

Biochemical Analysis

Cholesterol and triglycerides serum concentration were determined by a cholorimetric method (Bioclin, Belo Horizonte, MG, Brazil). 17β-Estradiol serum concentration was determined by radioimunoassay, using a commercial kit (Solid Phase Component System, INC Pharmaceuticals). The sensitivity of the kit was 0.13 pg/dl, and the intra and inter-assay variation coefficients were of 5.5% and 5.3%, respectively.

Statistical Analysis

The data are reported as mean ± standard deviation of eight animals. Statistical significance of experimental observations was determined by ANOVA, followed by Newman Keuls pos + hoc test. The level of significance was set at $P < 0.05$ (Sokal and Rohlf, 1995).

RESULTS

The chemical composition of diets is shown in Table 1. The data related to the total and individual isoflavone components of diets as daidzein, genistein, daidzin, glicitin, and genistin are shown in Table 2. There was no significant difference in the isoflavone components of diets. The food consumption per 100 g of body weight was the same among the groups.

Table 3 shows the body and organs weights, cholesterol, triglycerides, and estradiol serum levels of all

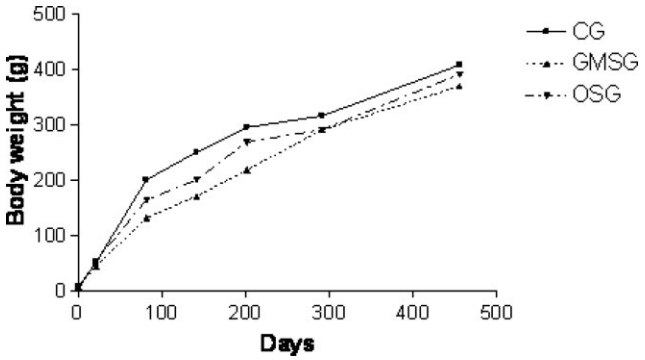

Fig. 1. Body weight from birth to adult age of the control group (CG), genetically modified soy group (GMSG) and organic soy group (OSG).

groups. Both OSG and GMSG groups had lower body weight when compared with CG, but this reduction was significant only in the GMSG ($P < 0.05$). There was no significant difference in the ovary or uterus absolute and relative weights (mg of tissue/g body weight) for the GMSG and OSG compared with the CG. Both GMSG and OSG groups demonstrated lower serum level of estradiol $P < 0.01$) than the CG. In relation to lipid profile, both GMSG and OSG groups demonstrated lower triglyceryde ($P < 0.05$) and cholesterol (CG vs. OSG = $P < 0.05$; CG vs. GMSG = $P < 0.01$) serum levels than the CG.

Body weight history from birth until adult age is shown in Fig. 1. Figure 2 shows the number of different classes of follicles and corpora lutea in the three examined groups. The number of primordial, primary,

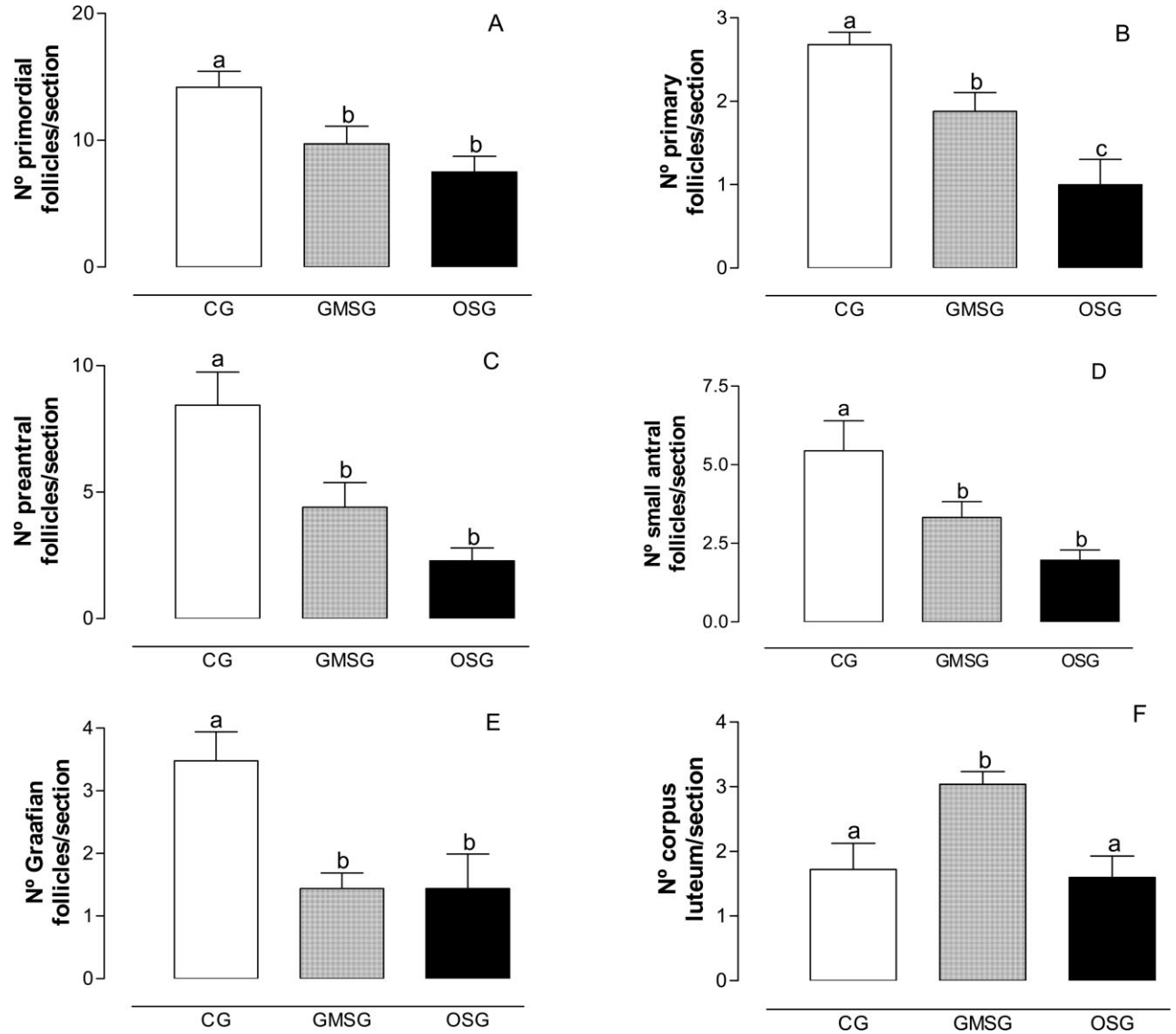

Fig. 2. The number of primordial (**a**), primary (**b**), preantral (**c**), small antral (**d**), graafian (**e**) follicles, and corpus luteum (**f**) in control group (CG), genetically modified soy group (GMSG) and organic soy group (OSG). Values are given as mean ± standard deviation of 8 animals. Different superscript letter means statistical significant differences.

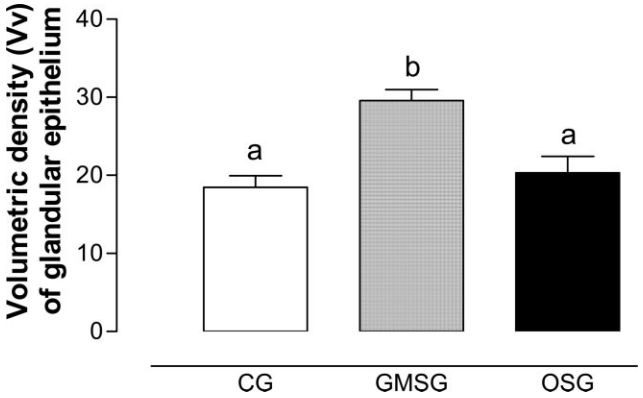

Fig. 3. Volumetric density of glandular epithelium of the control group (CG), genetically modified soy group (GMSG) and organic soy group (OSG). Values are given as mean ± standard deviation of eight animals. Different superscript letter means statistical significant differences.

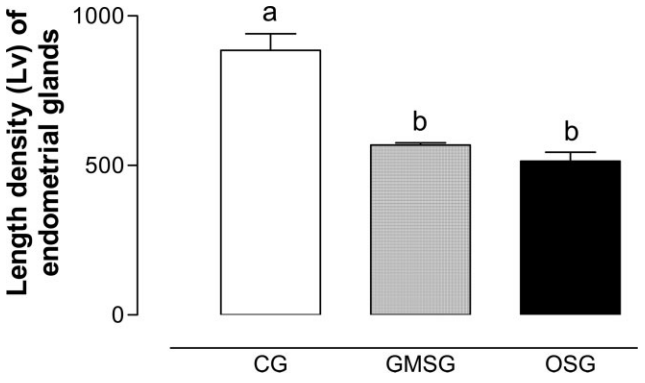

Fig. 4. Length density of endometrial glands of the control group (CG), genetically modified soy group (GMSG) and organic soy group (OSG). Values are given as mean ± standard deviation of eight animals. Different superscript letter means statistical significant differences.

preantral, small antral, and Graafian follicles was significantly reduced in both GMSG ($P < 0.05$) and OSG ($P < 0.01$) groups when compared with the CG. The number of corpus luteum was significantly ($P < 0.05$) increased only in the GMSG when compared with the CG. The number of primary follicles and corpus luteum was significantly different ($P < 0.05$) between the GMSG and OSG groups.

The morphometric analysis of the uterus showed that volumetric density of epithelium was significantly higher in the GMSG compared with both CG ($P < 0.01$) and OSG ($P < 0.05$) (Fig. 3). Both soy groups presented a significant reduction in the length density of endometrial glands ($P < 0.01$) when compared with the CG (Fig. 4).

Figure 5 show histological sections of ovary (A, B, and C) in CG, GMSG and OSG groups, respectively. Primary follicles consist of an oocyte surrounded by a single layer of cuboidal granulosa cells. The preantral follicles present a central oocyte surrounded by several layers of granulosa cells and bounded by thecal cells, which form a fibrous theca externa and an inner theca interna with no antrum. In antral follicles, fluid appeared between the granulosa cells, and the drops coalesced to form follicular fluid within the follicular antrum. In Graafian follicles, the follicular antrum is clearly developed, leaving the oocyte surrounded by a distinct and denser layer of granulosa cells, the cumulus oophorus. The corpus luteum is formed by luteal cells and abundant capillaries.

Figure 6 show histological sections of uterus (A, B, and C) in CG, GMSG, and OSG groups, respectively. The endometrial and glandular epithelium, stroma, and myometrium can be observed in the photomicrographs.

DISCUSSION

Soyfood has been reported to have beneficial effects including improving the lipid profile (Simons et al., 2000; Dent et al., 2001; Gardner et al., 2001; Kang et al., 2002; Engelman et al., 2005; Ho et al., 2007), bone metabolism (Marini et al., 2007; Ma et al., 2007), cancer development (Jin and MacDonald, 2002; Liu et al., 2005), without having any effects on the uterus and ovary in postmenopausal women or menopausal animals models (Bahr et al., 2005; Castillo et al., 2006; Kaari et al., 2006; Wood et al., 2006; Marini et al., 2007). However, if soyfood is used for neonatal, young, or adult animals at a reproductive age, it can cause adverse effects related to the reproductive organs (Jefferson et al., 2002, 2005, 2006; Michael et al., 2006; Piotrowska et al., 2006; Rachon et al., 2007a,b). On the basis of the soy consumption increment, this study was designed to compare the effects of a prolonged use of organic and transgenic soy on the lipid profile and ovary and uterus morphology.

Although the use of genetically modified food is still questionable, there is no evidence that genetic modification through biotechnology will impose immediate significant risks as food allergen sources beyond that of our daily dietary intake of foods from crop plants (Helm, 2003) or beyond other methodologies widely accepted in the food industry (Lack, 2002). Also, there is no evidence suggesting that recombinant DNA would be processed in the gut in any manner different from endogenous feed-ingested genetic material (Jennings et al., 2003; Sharma et al., 2006). The data presented here show that the effects of organic and transgenic soy consumption were very similar, except those observed in the corpora lutea and volumetric density of glandular epithelium.

In agreement with the literature, both organic and transgenic soy reduced the body weight (Demonty et al., 2002; Rachon et al., 2007a) and estradiol serum levels (Lu et al., 1996; Nagata et al., 1997; Duncan et al., 1999; Wood et al., 2007). Both soy treatments also improved the lipid profile by reducing cholesterol and triglycerides serum levels (Simons et al., 2000; Dent et al., 2001; Gardner et al., 2001; Kang et al., 2002; Engelman et al., 2005; Ho et al., 2007). Probably the reduction in estradiol serum levels reflects the capacity of isoflavones to bind the estrogen receptor and blocking the actions of endogenous estrogens (Lissin and Cooke, 2000). The alterations presented here were more marked in the transgenic group, which showed the lowest body weight and cholesterol and triglycerides levels.

Also, corroborating previous results (Jefferson et al., 2002, 2005, 2006; Michael et al., 2006; Piotrowska et al., 2006; Rachon et al., 2007a,b), both transgenic and

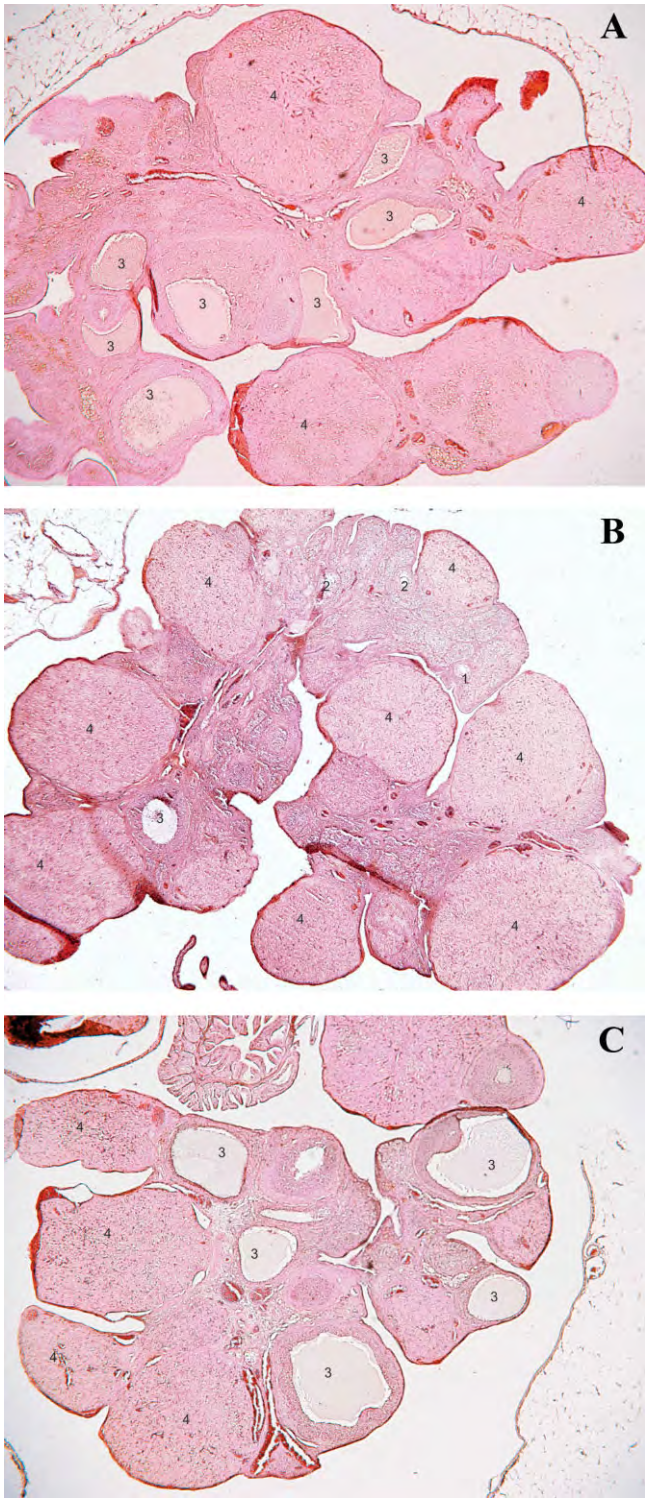

Fig. 5. (**A**) Photomicrograph showing ovary of control group (CG). 3, Graafian follicles; 4, corpus luteum. Magnification ×40. (**B**) Photomicrograph showing ovary of genetically modified soy group (GMSG). 1: preantral follicles; 2: antral follicles; 3: Graafian follicles; 4: corpus luteum. Magnification ×40. (**C**) Photomicrograph showing ovary of organic soy group (OSG). 3: Graafian follicles; 4: corpus luteum. Magnification ×40.

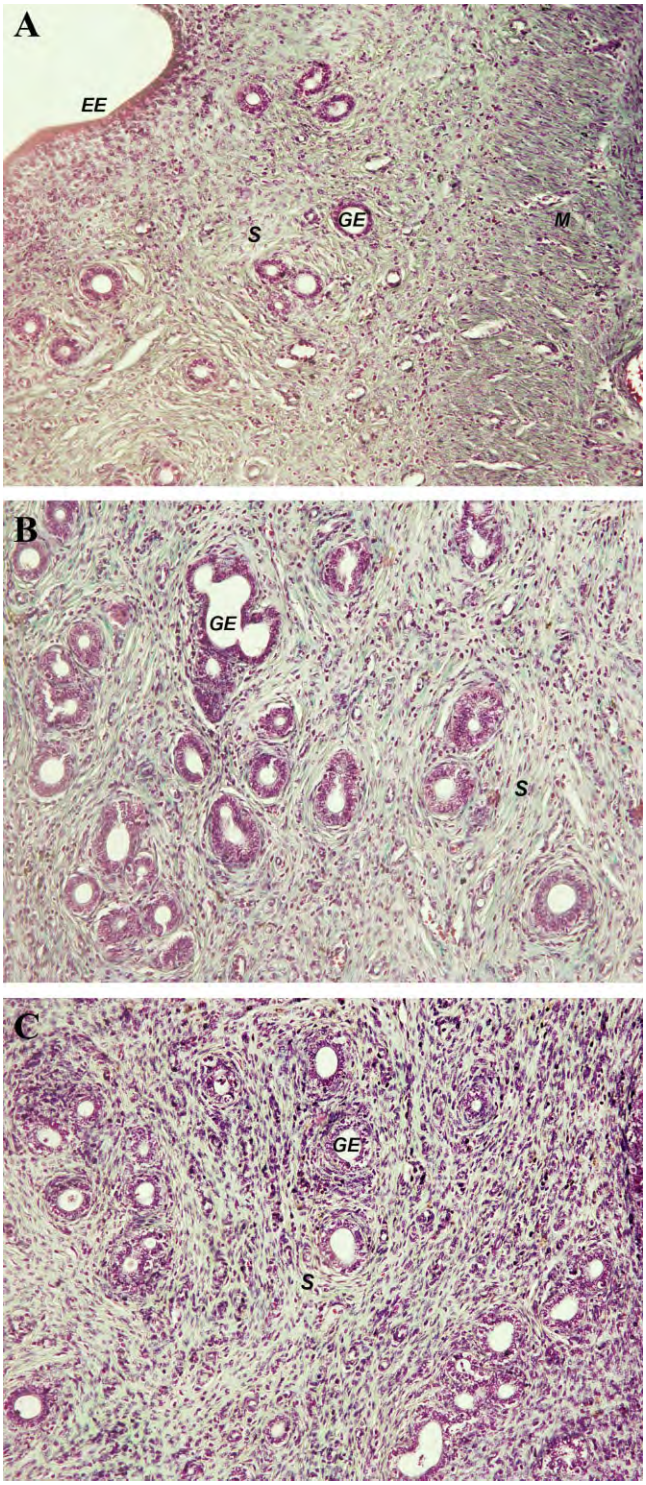

Fig. 6. (**A**) Photomicrograph showing uterus of control group (CG). EE, endometrial epithelium; GE, glandular epithelium; S, stroma; M, myometrium. Magnification ×100. (**B**) Photomicrograph showing uterus of genetically modified soy group (GMSG) GE, glandular epithelium; S, stroma. Magnification ×100. (**C**) Photomicrograph showing uterus of organic soy group (OSG). GE, glandular epithelium; S, stroma. Magnification ×100.

organic soy had an adverse effect on some parameters of the uterus and ovary morphology. The number of the growing follicles was significantly reduced in both soy-treated groups, in spite of normal corpus luteum number in the organic group. In relation to the uterus, both soy-treated groups exhibited a reduction in the length density of the glands, while the volumetric density of the epithelium was unaltered in the organic group. These data suggest that the transgenic and organic soy may have specific effects in the reproductive system.

The isoflavone content of both transgenic and organic soy was evaluated and no significant difference in the individual components or in food consumption was found. So, at this moment we may assume that the differences related to the transgenic and organic soy are not related to isoflavone, but it can probably be related to the small differences in fat, sugar and especially protein or amino acids diet content among the three groups.

In summary, both transgenic and organic-derived soy diets improved the lipid profile and reduced body weight; however, alterations in uterine and ovarian morphology were also found in animals with prolonged exposure to these diets. The prolonged use of soy-based diets and their relation to reproductive health warrants further investigation.

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Medizinisches Labor Bremen

Gemeinschaftspraxis für
Laboratoriumsmedizin, Mikrobiologie
und Infektionsepidemiologie, Biochemie
Umweltmedizin

Haferwende 12 • 28357 Bremen

Document Title	**Determination of Glyphosate residues in human urine samples from 18 European countries**
Test Compound	**Glyphosate and AMPA**
Study Initiation Date	**March 2013**
Study Completion Date	**June 6, 2013**
Test Facility	**Medical Laboratory Bremen, Haferwende 12, 28357 Bremen, Germany**
Sponsor	**BUND, FoE**
Date of the Document	**June 12, 2013**

STUDY LOCATION

The preparation of standards and analyses of samples were carried out at the Test Facility:

Medical Laboratory Bremen,
Haferwende 12,

28357 Bremen, Germany

Under the responsibility of the Study Director, Dr. Hans-Wolfgang Hoppe, according to the relevant Operating Procedures.

Contact Dr. Hoppe:

0049 (0) 421 2072 -251

Hans-Wolfgang.Hoppe@mlhb.de

The analytical phase of the study was started on March 28, 2013 and was completed June 6, 2013.

The hardcopy raw data will be scanned and stored as electronic media and kept at Bremen Lab for a period of at least 1 year. A copy of the electronic media and the original hardcopy raw data will be sent to sponsor for archival purposes on demand.

REPORT APPROVAL

TEST FACILITY

Medical Laboratory Bremen,
Haferwende 12,
28357 Bremen, Germany

	Signature	Date
Dr. rer. nat. Hans-Wolfgang Hoppe Study Director	H. W. Hoppe	6.6.2013

TABLE OF CONTENTS

1. OBJECTIVE

Determination of Glyphosate and AMPA residues in human urine samples. The goal of this study was to support the biomonitoring work of the BUND / FoE against the background of increasing Glyphosate use in some European countries.

2. Management of Biological Samples

We received shipments from 18 European countries during the period of March 22 (Belgium) to May 21 (Spain, Poland). Each shipment included 8-12 samples. The Spanish samples made a detour via Budapest. All urines were ice cold and in a good condition at receipt and were stored at –18°C until date of sample work-up. At reception, MLHB sent a confirmation of receipt to the Sponsor and has checked for any mismatch between the shipment and the list provided by the Sponsor. On completion of the assays, the samples were kept deep frozen. The study samples will be stored for a period of 1 year.

3. Analytical procedures

The human urine samples were analyzed by means of a validated GC-MSMS method. In addition we determined creatinine to correct for diurese, if needed. Brief descriptions and the relevant specifications are listed below.

Analytical Method for Glyphosate and AMPA

Sample preparation:
1) Evaporation and dissolution of the residue in methanol,
2) derivatization using trifluoroethanol and trifluoroactic acid anhydride

Internal Standards:
$^{13}C_2{}^{15}N$-Glyphosate; $^{13}C^{15}N$-AMPA

Measurement: GC-MSMS, NCI mode
Capillary Column: HP-Innowax

Precision: 8% each
Recovery: 95% each
Linearity: 0,2 - 20 µg/L each
Limit of Quantitation (LOQ): 0,15 µg/L each

Figure 1 shows a typical chromatogram of a processed native urine sample.

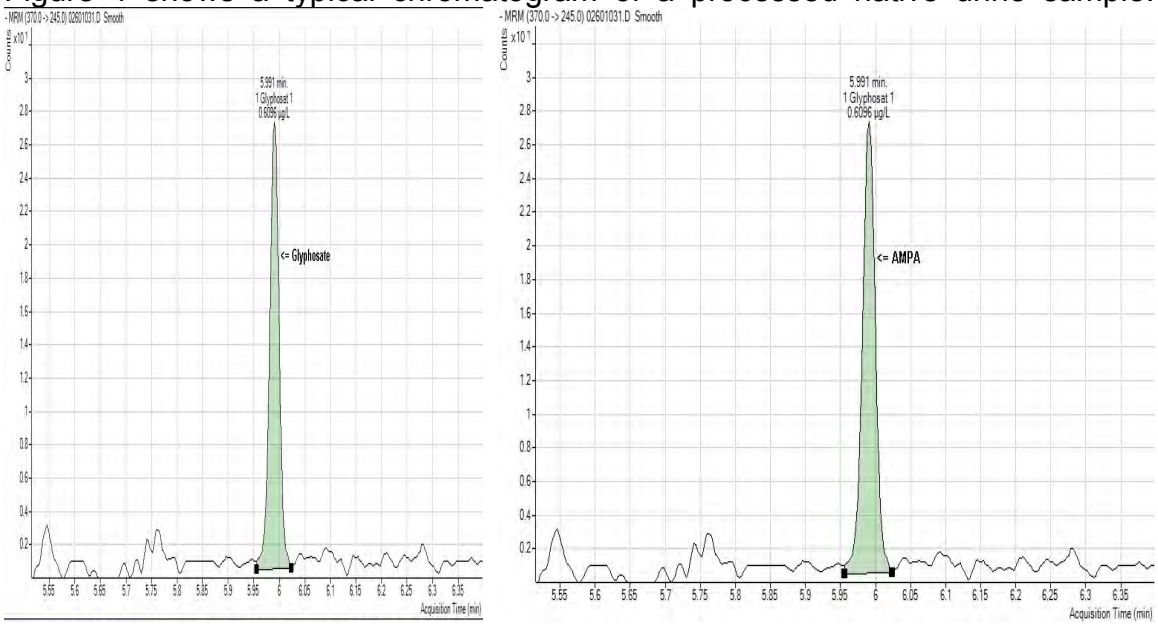

Fig. 1. GC-MSMS Chromatogramm of a processed urine sample (Belgium 11). The concentrations were as follows: Glyphosate: 0,6 µg/L; AMPA: 0,4µg/L

Method for Creatinine in urine: Jaffé-Reaction

4. Reference Values

Table 1: Reference values

Parent compound	Biomarker	Reference Value	Literature
Glyphosate	Glyphosate	0,8 µg/L	Empirical value, MedLab 2012
Glyphosate	AMPA	0,5 µg/L	Empirical value, MedLab 2012
Creatinine	Creatinine	Range 0,3-3 g/L	

The reference values for Glyphosate and AMPA are only tentative. They were derived from an urban collective (n=90) and are defined as the 95. percentile of the measured values. They were established by *Medical Laboratory Bremen* in 2012 during the process of the method validation. Strictly speaking they are only valid to the region of Bremen.

5. Assay Results

A total of 182 humane urine samples were analyzed for residues of Glyphosate and the metabolite AMPA using a new GC-MSMS method. The results are presented in table 2. The samples to be analyzed were distributed across 6 runs. Besides the EU-Samples each run contained 2 QC-samples with known

Glyphosate and AMPA concentrations. Creatinine concentrations were only used to check whether the spot urine samples are valid. All creatinine levels are inside the reference range (see table 2). The analytical results of Glyphosate and AMPA are not creatinine corrected. A mean blank value of 0.03 µg/L was subtracted from the measured concentrations of the urine samples and controls (Table 2 and 3).
Quality control samples showed good precision and accuracy throughout the measurement of the study samples as an indication of the good method performance (see table 3). A brief statistical evaluation including frequency of detection, mean and maximum values sorted by EU countries is given in table 4.

Table 2: Glyphosate and AMPA concentrations in human urine samples

Participant	Glyphosate	AMPA	Creatinine
	µg/L	µg/L	g/L
Belgium no 2	<0,15	0,195	0,43
Belgium no 3	<0,15	<0,15	0,9
Belgium no 4	0,256	0,197	2,27
Belgium no 5	<0,15	<0,15	0,81
Belgium no 6	0,17	1,256	1,84
Belgium no 7	0,18	0,684	1,64
Belgium no 8	0,190	<0,15	1,03
Belgium no 9	<0,15	<0,15	2,22
Belgium no 10	0,211	<0,15	1,28
Belgium no 11	0,575	0,373	2,7
Belgium no 12	<0,15	<0,15	0,98
Latvia No 1	<0,15	<0,15	0,79
Latvia No 2	<0,15	<0,15	0,96
Latvia No 3	0,896	0,391	1,9
Latvia No 4	0,208	0,150	2,33
Latvia No 5	<0,15	<0,15	1,95
Latvia No 6	1,821	0,15	2,75
Latvia No 7	0,636	0,706	2,01
Latvia No 8	0,203	0,220	2,28
Latvia No 9	0,339	<0,15	3,35
Latvia No 10	<0,15	<0,15	1,62
Latvia No 11	<0,15	<0,15	0,36
UK No 1	1,636	0,560	2,25
UK No 2	0,261	<0,15	0,7
UK No 3	0,205	<0,15	0,62

UK No 4	<0,15	<0,15	0,59
UK No 5	<0,15	<0,15	0,83
UK No 6	1,068	0,364	1,5
UK No 7	<0,15	<0,15	0,87
UK No 8	0,264	0,241	1,64
UK No 9	0,579	0,483	0,57
UK No 10	0,425	0,239	0,92
France No 1	<0,15	<0,15	1,65
France No 2	<0,15	<0,15	2,26
France No 3	<0,15	<0,15	0,96
France No 4	0,209	0,281	2,16
France No 5	0,200	0,408	2,4
France No 6	<0,15	0,209	1,19
France No 7	<0,15	<0,15	0,34
France No 8	<0,15	<0,15	1,33
France No 9	<0,15	<0,15	2,23
France No 10	0,232	<0,15	2,64
Czech Republic No 1	0,302	0,217	1,16
Czech Republic No 2	0,916	0,296	2,33
Czech Republic No 3	<0,15	<0,15	0,25
Czech Republic No 4	<0,15	<0,15	0,6
Czech Republic No 5	0,273	0,192	0,88
Czech Republic No 6	<0,15	<0,15	0,63
Czech Republic No 7	0,247	0,208	1,15
Czech Republic No 8	0,191	<0,15	1,07
Czech Republic No 9	0,159	0,212	0,52
Czech Republic No 10	<0,15	<0,15	0,34
Bulgaria No 1	<0,15	<0,15	0,96
Bulgaria No 2	<0,15	<0,15	0,96
Bulgaria No 3	<0,15	<0,15	0,75
Bulgaria No 4	<0,15	<0,15	1,04
Bulgaria No 5	0,176	<0,15	2,82
Bulgaria No 6	<0,15	<0,15	0,46
Bulgaria No 7	<0,15	0,201	1,53
Bulgaria No 8	<0,15	0,166	0,89
Bulgaria No 9	<0,15	<0,15	1,82
Bulgaria No 10	<0,15	<0,15	1,3
Malta No 1	0,363	0,180	2,62
Malta No 2	0,293	<0,15	1,08
Malta No 3	0,906	0,644	1,42
Malta No 4	1,555	0,886	2,17
Malta No 5	0,379	0,267	1,38
Malta No 6	1,242	0,387	1,19

Malta No 7	<0,15	<0,15	1,3
Malta No 8	0,992	0,397	2,6
Malta No 9	1,290	0,580	1,51
Malta No 10	1,127	0,552	1,52
Macedonia No 1	<0,15	<0,15	1,97
Macedonia No 2	<0,15	<0,15	1,63
Macedonia No 3	<0,15	<0,15	1,56
Macedonia No 4	0,239	<0,15	0,4
Macedonia No 5	<0,15	<0,15	0,96
Macedonia No 6	<0,15	<0,15	1,23
Macedonia No 7	<0,15	<0,15	1,72
Macedonia No 8	<0,15	<0,15	1,65
Macedonia No 9	<0,15	<0,15	3,15
Macedonia No 10	<0,15	<0,15	1,67
Austria No 1	0,198	<0,15	2,73
Austria No 2	<0,15	0,163	1,81
Austria No 3	<0,15	<0,15	2,97
Austria No 4	<0,15	<0,15	1,19
Austria No 5	<0,15	<0,15	0,33
Austria No 6	<0,15	<0,15	3,22
Austria No 7	<0,15	<0,15	1,15
Austria No 8	<0,15	<0,15	0,23
Austria No 9	<0,15	<0,15	0,28
Austria No 10	0,153	0,156	1,17
Croatia No 1	<0,15	<0,15	0,79
Croatia No 2	<0,15	<0,15	2,07
Croatia No 3	0,224	2,630	1,9
Croatia No 4	0,187	<0,15	1,57
Croatia No 5	<0,15	<0,15	1,65
Croatia No 6	0,158	<0,15	0,71
Croatia No 7	<0,15	<0,15	0,69
Croatia No 8	<0,15	<0,15	1,07
Croatia No 9	<0,15	<0,15	2,1
Croatia No 10	0,424	<0,15	1,02
Hungary No 1	<0,15	<0,15	0,9
Hungary No 2	<0,15	<0,15	1,13
Hungary No 3	<0,15	0,270	1,2
Hungary No 4	0,176	<0,15	1,21
Hungary No 5	0,171	0,153	0,49
Hungary No 6	0,171	<0,15	2,1
Hungary No 7	<0,15	<0,15	1,66
Hungary No 8	<0,15	<0,15	0,72
Hungary No 9	<0,15	<0,15	1,11

Hungary No 10	<0,15	<0,15	0,8
Switzerland 1	<0,15	<0,15	0,67
Switzerland 2	<0,15	<0,15	0,99
Switzerland 3	<0,15	<0,15	0,78
Switzerland 4	<0,15	<0,15	1,22
Switzerland 5	<0,15	<0,15	1,09
Switzerland 6	<0,15	<0,15	0,73
Switzerland 7	0,156	<0,15	1,9
Switzerland 8	<0,15	<0,15	1,57
Switzerland 9	0,159	<0,15	2,35
Switzerland 10	<0,15	<0,15	0,56
Switzerland 11	<0,15	<0,15	2,41
Switzerland 12	<0,15	<0,15	0,74
Netherlands 1	<0,15	<0,15	4,19
Netherlands 2	0,156	0,245	2,61
Netherlands 3	<0,15	<0,15	1,13
Netherlands 4	1,016	0,498	2,23
Netherlands 5	0,159	0,172	1,71
Netherlands 6	0,429	0,640	1,99
Netherlands 7	<0,15	<0,15	1,45
Netherlands 8	0,701	0,256	2,05
Germany 1	0,238	0,228	0,48
Germany 2	<0,15	<0,15	0,79
Germany 3	0,209	0,213	0,83
Germany 4	0,486	0,439	1,9
Germany 5	<0,15	<0,15	0,46
Germany 6	<0,15	0,202	1,7
Germany 7	0,460	<0,15	1,72
Germany 8	0,226	<0,15	1,38
Germany 9	0,445	0,700	1,64
Germany 10	0,204	0,205	2,6
Cyprus 1	0,199	<0,15	1,32
Cyprus 2	<0,15	<0,15	0,86
Cyprus 3	0,158	<0,15	2,33
Cyprus 4	<0,15	<0,15	0,65
Cyprus 5	0,180	0,674	0,99
Cyprus 6	0,223	0,228	2,01
Cyprus 7	<0,15	<0,15	0,85
Cyprus 8	<0,15	0,643	2,05
Cyprus 9	<0,15	<0,15	1,86
Cyprus 10	0,250	0,584	1,61
Georgia 1	<0,15	<0,15	1,91
Georgia 2	0,193	0,176	1,7

Georgia 3	0,353	0,178	1,61
Georgia 4	<0,15	<0,15	1,31
Georgia 5	<0,15	<0,15	0,81
Georgia 6	<0,15	<0,15	1,62
Georgia 7	<0,15	<0,15	1,5
Georgia 8	<0,15	0,185	1,6
Georgia 9	<0,15	<0,15	0,96
Georgia 10	<0,15	<0,15	0,62
Spain 1	<0,15	<0,15	1,25
Spain 2	<0,15	<0,15	0,74
Spain 3	0,160	<0,15	1,54
Spain 4	0,221	0,820	2,31
Spain 5	<0,15	<0,15	0,43
Spain 6	<0,15	<0,15	0,17
Spain 7	0,168	0,165	1,36
Spain 8	0,175	<0,15	0,68
Spain 9	<0,15	<0,15	0,76
Spain 10	<0,15	0,160	1,46
Poland 1	0,235	0,206	1,96
Poland 2	<0,15	<0,15	0,85
Poland 3	<0,15	<0,15	0,34
Poland 4	0,763	0,285	0,55
Poland 5	0,233	0,267	1,42
Poland 6	0,377	0,406	1,36
Poland 7	0,528	0,237	0,94
Poland 8	0,599	0,283	2,9
Poland 9	0,168	<0,15	0,91
Poland 10	<0,15	<0,15	0,92

Table 3: Results of quality controls

Run	Glyphosate	AMPA	Glyphosate	AMPA
Target (µg/L)	5	5	1	0,5
	Conc (µg/L)	Conc (µg/L)	Conc (µg/L)	Conc (µg/L)
Run 1	4,76	5,14	0,95	0,53
Run 2	5,10	5,48	1,18	0,50
Run 3	5,27	5,33	1,02	0,47
Run 4	5,35	5,29	1,02	0,47
Run 5	5,50	5,40	1,06	0,55
Run 6	5,30	5,10	1,04	0,53

Mean	5,21	5,29	1,05	0,51
CV (%)	4,52	2,55	7,34	6,76
Accuracy (%)	104,24	105,79	104,54	101,04

Table 4: Statistics
To calculate the mean values, results below the LOQ (0,15 µg/L) are replaced with
½ LOQ (0,075 µg/L).

EU country		Subjects	Glypho Frequency of Detektion	Glypho Frequency of Detektion	Glypho Mean	Glypho Maximum Value	AMPA Frequency of Detektion	AMPA Frequency of Detektion	AMPA Mean	AMPA Maximum Value
		n	n	%	µg/L	µg/L	n	%	µg/L	µg/L
Belgium	B	11	6	54,55	0,18	0,57	5	45,45	0,29	1,26
Latvia	LV	11	6	54,55	0,41	1,82	5	45,45	0,19	0,71
Great Britain	GB	10	7	70,00	0,47	1,64	5	50,00	0,23	0,56
France	F	10	3	30,00	0,12	0,23	3	30,00	0,14	0,41
Czech Republic	CZ	10	6	60,00	0,24	0,92	5	50,00	0,15	0,30
Bulgaria	BG	10	1	10,00	0,09	0,18	2	20,00	0,10	0,20
Malta	M	10	9	90,00	0,82	1,56	8	80,00	0,40	0,89
Macedonia	MK	10	1	10,00	0,09	0,24	0	0,00	0,08	0,08
Austria	A	10	2	20,00	0,10	0,20	2	20,00	0,09	0,16
Croatia	HR	10	4	40,00	0,14	0,42	1	10,00	0,33	2,63
Hungary	H	10	3	30,00	0,10	0,18	2	20,00	0,10	0,27
Switzerland	CH	12	2	16,67	0,09	0,16	0	0,00	0,08	0,08
Netherlands	NL	8	5	62,5	0,34	1,02	5	62,50	0,25	0,64
Germany	D	10	7	70,00	0,25	0,49	6	60,00	0,23	0,70
Cyprus	CY	10	5	50,00	0,14	0,25	4	40,00	0,26	0,67
Georgia	GE	10	2	20,00	0,11	0,35	3	30,00	0,11	0,19
Spain	E	10	4	40,00	0,12	0,22	3	30,00	0,17	0,82
Poland	PL	10	7	70,00	0,31	0,76	6	60,00	0,20	0,41
Total		182	80	43,9	0,21	1,56	65	35,71	0,18	2,63

6. Conclusions

In this study, 182 urine samples received from 18 European countries were analyzed for Glyphosate and AMPA residues using a new GC-MSMS method (see table 2). With a LOQ of 0,15 µg/l, on average 44 % and 36 % of the urine samples analyzed were found to contain quantifiable levels of Glyphosate and AMPA, respectively. However the frequency of detection calculated for each individual EU-state ranged from 10% to 90% (see Table 4). The highest Glyphosate concentration was 1,8 µg/L (Latvia 6), the highest AMPA concentration was 2,6 µg/L (Croatia 3). All in all 12 (6,6%) participants of the study significantly exceeded the tentative reference value of 0,8 µg/L for Glyphosate (see section 4).

In general, Glyphosate and AMPA urinary level do not correlate very well. This is due to the finding that the ratio AMPA/Glyphosate (AGR) in human urine is very variable probably reflecting the variable AGRs in diet. A high AGR suggests an additional exposure against Aminopolyphosphonate based tensides like ATMT or EDTMP, which easily degrade to AMPA.

The results give a first idea to which extent adults in 18 European countries are exposed to Glyphosate. The regional and individual variations are large. Diet seems to be the main sources of exposure. However, more scientific work is needed to distinguish between different exposure situations.

A long-term toxicology study on pigs fed a combined genetically modified (GM) soy and GM maize diet

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Abstract

A significant number of genetically modified (GM) crops have been approved to enter human food and animal feed since 1996, including crops containing several GM genes 'stacked' into the one plant. We randomised and fed isowean pigs (N=168) either a mixed GM soy and GM corn (maize) diet (N=84) or an equivalent non-GM diet (N=84) in a long-term toxicology study of 22.7 weeks (the normal lifespan of a commercial pig from weaning to slaughter). Equal numbers of male and female pigs were present in each group. The GM corn contained double and triple-stacked varieties. Feed intake, weight gain, mortality and blood biochemistry were measured. Organ weights and pathology were determined post-mortem. There were no differences between pigs fed the GM and non-GM diets for feed intake, weight gain, mortality, and routine blood biochemistry measurements. The GM diet was associated with gastric and uterine differences in pigs. GM-fed pigs had uteri that were 25% heavier than non-GM fed pigs (p=0.025). GM-fed pigs had a higher rate of severe stomach inflammation with a rate of 32% of GM-fed pigs compared to 12% of non-GM-fed pigs (p=0.004). The severe stomach inflammation was worse in GM-fed males compared to non-GM fed males by a factor of 4.0 (p=0.041), and GM-fed females compared to non-GM fed females by a factor of 2.2 (p=0.034).

Key words: GMO**,** GM corn, GM soy, GM animal feed, toxicology, stomach inflammation, uterus weight.

Introduction

Genetically modified (GM) crops have entered human food and animal feed in increasing amounts since they were commercially released into fields in the USA in 1996 (USDA, 2011). The main traits in GM crops to date have been to express proteins for herbicide tolerance (Ht) and insect resistance (Carman, 2004; USDA, 2011). Herbicide tolerant crops are engineered to produce one or more proteins that allow the crop to survive being sprayed with a given herbicide. Insect resistant crops are usually engineered to produce

one or more insecticidal proteins that are toxic to target insects. The latter proteins are usually Bt proteins, so named because they are structurally similar to naturally-occurring Cry proteins from a soil bacterium, *Bacillus thuringiensis* (ANZFA, NDb). Hence these crops are also called Bt crops.

Of the GM crops planted in the USA, herbicide-tolerant GM soy has been widely adopted and now constitutes 94% of the soy planted in the USA (USDA, 2011). GM corn varieties have also been widely adopted in the USA (USDA, 2011). They usually contain Ht or Bt traits, or a 'stacked' combination of them (Pioneer Hi-Bred, 2012).

Prior to the release of a new GM crop into the food supply, the developer provides food regulators in many countries with studies it has done on the crop. These studies often include animal feeding studies, even though some regulators, such as Australia's, do not require them (FSANZ, ND; Carman, 2004), while the USA has a voluntary system. Many food regulators do not require any studies to be done on crops containing several "stacked" genes if all the genes in the stack have previously been individually approved for use in the same kind of plant (EFSA, 2010; FSANZ, 2010). Consequently, safety studies on stacked crops are less frequent, even though an analysis of official data (USDA, 2011) indicates that over 37% of GM corn varieties currently planted in the USA are stacked with both Ht and Bt traits.

There have been a number of reviews of the published literature on the safety of GM crops. For example, Flachowsky et al. (2005) and Preston (2005) both conducted reviews and both concluded that GM crops were safe for animals and people to eat. However, many of the feeding studies reviewed used non-mammals (e.g. birds, fish) or animals were fed the crop in a form that humans do not eat (e.g. silage) or only animal production outcomes were measured such as body weight, carcass weight, breast meat yield or milk production, which may not be indicative of potential human health outcomes (Carman, 2004). Only a small proportion of published animal feeding studies have been longer-term toxicological studies where a GM crop was fed to animals that are physiologically comparable to humans, and organs, blood and tissue samples were taken from the animals and examined to assess if the crop caused any adverse effects.

Two recent reviews of these rarer toxicology-type studies have recently been published. Snell et al. (2011) reviewed 12 studies of 90 days or longer duration and concluded that GM plants were nutritionally equivalent to non-GM plants and could be safely used in food and feed. However, once again, most of the studies reviewed used animals that were either not physiologically comparable to humans, or used only small numbers of animals. A broader picture is given in a series of three reviews by Domingo (2000; 2007) and Domingo & Bordonaba (2011). The first two papers concluded that there were few published studies investigating toxicology or health risks, while the third found that most of the more recent studies concentrate on only a few GM crops (soy, corn and rice), ignoring many other GM crops such as potatoes, peas and tomatoes.

Another review of 19 studies of mammals fed GM soy or maize has recently been conducted (Séralini et al., 2011). These authors also reviewed the raw data of some other authors' 90-day feeding studies. They found some evidence for adverse liver and kidney effects from eating some GM crops and concluded that 90-day feeding studies were insufficient to evaluate chronic toxicity of GM crops.

More recently, a highly publicised (e.g. Poulter, 2012), much longer study of two-years' duration on NK603 herbicide-tolerant corn (which contains one of the genes fed in the present study) has been published (Séralini et al. 2012). There were indications of higher death rates, more tumours and liver and kidney pathologies in GM-fed rats.

The aim of the present study was to perform a thorough, long-term toxicology study (for 22.7 weeks, being the normal lifespan of a commercial pig from weaning to slaughter) on pigs in a USA commercial piggery in order to compare the effects of eating either a mixed GM soy and GM corn diet, or an equivalent diet with non-GM ingredients. Pigs in the USA are usually fed a mixed corn and soy diet, containing a high proportion of GM varieties. Even though pigs are physiologically similar to humans, particularly for gastrointestinal observations, very few toxicology studies have been conducted on them for GM crops (Walsh et al., 2012a). In doing this study, we not only used animals that were physiologically similar to humans, but we also weighed and internally examined organs and took blood for biochemical analysis. We further used a large enough sample size (168 pigs, 84 per group) to be able to determine statistical significance for key toxicological outcomes. We also used GM crops that are planted in significant quantities in the USA (Ht soy, and Ht and Bt corn) and hence are commonly eaten by pigs and humans in the USA. We further fed these crops as a mixed diet. Mixed diets commonly occur for pigs and humans. This study therefore reflects the effects of eating GM crops in the ‘real world’. To our knowledge, this is the first study of its kind conducted.

Materials and Methods

Animal feed

In accordance with usual commercial USA piggery practice, soy and corn were obtained direct from farmers who had grown it commercially. Different GM corn varieties are usually co-mingled in farm storage. The corn used in this study contained 90% DK 42-88 RR YG PL (a triple stack of NK603, MON863 and MON810 genes) with the remainder being equal quantities of Pannar 5E-900RR (containing NK603), Pannar 4E-705RR/Bt (a double stack of NK603 and MON810) and Producers 5152 RR (containing NK603). Therefore, the GM corn that was used was genetically modified to produce three new proteins. Two were Bt proteins that protected the plant against insect attack, while the third protein provided the plant with tolerance to the herbicide glyphosate (Testbiotech, 2012; Monsanto, 2012).

Because Roundup Ready™ (RR) soy is predominant in the GM soy market, this was used. This crop contains a gene that provides tolerance to the herbicide glyphosate. GM DNA analysis (Genetic ID, Fairfield, Iowa, US) confirmed that the GM corn contained a combination of NK603, MON863 and MON810 genes (expressing the CP4 EPSPS, Cry 3Bb1 and Cry 1Ab proteins respectively), that the RR soy was 100% RR soy (expressing the CP4 EPSPS protein), that the non-GM feed contained a median of 0.4% GM corn and that the non-GM soy contained a median of 1.6% GM soy. Such GM contamination of apparent non-GM material is common in the US.

In a similar way to the GM crops used, non-GM soy and non-GM corn were also obtained direct from farmers who had grown it commercially for human food and animal feed. Isogenic parental varieties of the GM crops, from which the GM crops were developed, were not used because they are generally not commercially available to buy. Furthermore, triple-stacked corn containing all three genes used here was developed

from conventionally cross-breeding several GM crops, each of which has a non-GM parent, leading to a multiplicity of isogenic parental varieties that would need to be used in combination for a control diet. As the aim of this study was to compare the effects of GM and non-GM varieties present in animal feed and human food in the real world, the soy and corn for the control diet was instead chosen as a mixture of non-GM soy and corn that was destined for animal feed and human food and that came from the same geographical area. The GM soy and corn used in this study have been determined to be compositionally and substantially equivalent to non-GM varieties of soy and corn by government regulators (ANZFA, 2002, NDa, NDb; FSANZ, 2003, 2006) which indicates that there should be no phenotypical variation between the GM and non-GM varieties used in this study that could influence the outcomes measured in this study.

GM and non-GM corn were both ground using the same cleaned equipment, size screen and revolutions per minute to obtain the same particle size. GM and non-GM soy beans were also processed on the same type of cleaned equipment - using Insta-Pro extruders and expellers, rather than being solvent-extracted, in order to preserve the identity of the beans during processing into soybean meal. This process purees the beans and squeezes out most of the oil, leaving a residual oil content of 8%. In the process, the beans are heated to 153^{o}C to 166^{o}C. As pigs grow, they require different amounts of nutrients, so six different sub-diets were progressively used. Soy content decreased from 26.5% to 13.0%, corn increased from 56.4% to 83.8% and protein decreased from 18.3% to 13.3% of the diet (Table 1). Ingredients, including supplements, were those routinely used by the piggery and were the same between groups. The GM and non-GM diets had the same protein, energy, macro- and micro-nutrient contents and only differed in the use of GM or non-GM soy and corn. Pigs were fed on a self-feeding, full-feed basis. The amount of feed consumed by each group was recorded.

Table 1. Details of the six body-weight-specific sub-diets used progressively as pigs grew.

	Sub-diet number					
	1	**2**	**3**	**4**	**5**	**6**
Pig weight (lb)[a]	14-25	25-60	60-90	90-130	130-200	200-260
No. days on diet[b]	39-40	17-18	23-24	24-25	37-38	15-17
Average daily intake (lb)	0.9	2.43	3.45	4.71	6.10	6.78
Protein (%)	18.6	18.0	17.4	16.3	15.2	14.7
Soy (%)[c]	26.5	25.0	23.4	20.4	17.5	16.0
Corn (%)[d]	70.0	71.6	73.2	76.3	79.8	81.3
UN premix (%)[e]	2.5	2.5	—	—	—	—
UG premix (%)[f]	—	—	2.5	2.5	—	—
UF premix (%)[g]	—	—	—	—	2.5	2.5
Boost premix (%)[h]	0.0025	0.0025	0.001	0.0015	0.0015	0.0015
Extra lysine	—	—	0.001	0.0005	—	—
Extra $CaCO_3$ (%)	0.0075	0.0075	0.006	0.006	0.002	0.002
200 mesh bentonite clay (%)	0.0035	0.0035	0.0035	0.0035	0.0035	0.0035

a As the piggery was in the USA, pig diets were changed when pigs reached a certain weight in pounds.

b Because pig handlers were required to keep to usual piggery practices and were blinded as to the GM feeding status of each group of pigs, pigs in each group were changed from one sub-diet to the next according to the body weight of the group. Consequently, one group was often changed to the next sub-diet a day before the other group. While the GM-fed group spent one day longer on a particular diet than the non-GM group for three diets, the non-GM group spent a day longer on a particular diet for the other three diets. Therefore, there was neither a trend nor a difference in the progression of the two groups from one diet to another. Pigs were fed for a total of 158 days if they were slaughtered on the first of the two slaughter days, and 159 days if they were slaughtered on the second slaughter day.

c GM soy went into the GM diets and non-GM soy into the non-GM diets.

d GM corn went into the GM diets and non-GM corn into the non-GM diets.

e Ultra Nursery Plus Premix from Advanced Biological Concepts, Osco, Illinois, containing (as copied directly from the label) guaranteed amounts of 0.5% crude protein, 6.0% lysine, 0.5% crude fat, 3.0% crude fiber 13.0% to 15% calcium, 13.0% phosphorus, 16.0% to 18.0% sodium chloride, 10ppm selenium, 1,500 ppm zinc, 190,000 IU/lb vitamin A, 25,000 IU/lb vitamin D_3 and 800 IU/lb vitamin E. Other ingredients on the label (not quantified), include: copper, iron, zinc, manganese, choline, ascorbic acid, niacin, riboflavin, pantothenic acid, vitamin K, vitamin B_{12}, carotene and iodine.

f Ultra Grower Premix Plus from Advanced Biological Concepts, Osco, Illinois, containing (as copied directly from the label) guaranteed amounts of 0.5% crude protein, 1.0% lysine, 0.5% crude fat, 3.0% crude fiber, 15.0% to 17% calcium, 12.0% phosphorus, 15.0% to 17.0% sodium chloride, 3ppm selenium, 1,500 ppm zinc, 160,000 IU/lb vitamin A, 22,000 IU/lb vitamin D_3 and 800 IU/lb vitamin E. Other ingredients on the label (not quantified) include: copper, iron, zinc, manganese, choline, niacin, riboflavin, pantothenic acid, vitamin K, vitamin B_{12}, carotene and iodine.

g Ultra Finisher Premix Plus from Advanced Biological Concepts, Osco, Illinois, containing (as copied directly from the label) guaranteed amounts of 0.5% crude protein, 3.0% lysine, 0.5% crude fat, 3.0% crude fiber, 18.0% to 20.0% calcium, 10.0% phosphorus, 6.5% to 7.5% sodium chloride, 3ppm selenium, 4,000 ppm zinc, 125,000 IU/lb vitamin A, 20,000 IU/lb vitamin D_3 and 500 IU/lb vitamin E. Other ingredients on the label (not quantified) include: copper, iron, zinc, potassium, magnesium, manganese, choline, ascorbic acid, niacin, riboflavin, pantothenic acid, vitamin K, vitamin B_{12}, carotene and iodine.

h Natural Boost from Advanced Biological Concepts, Osco, Illinois, containing (as copied directly from the label) guaranteed amounts of 10.0% crude protein, 0.005% lysine, 0.005% methionine, 1.0% crude fat, 24.0% crude fiber, 40.0% acid detergent fiber, 0.2% to 0.7% calcium, 0.2% phosphorus, 1.0% to 1.5% sodium chloride, 0.5% potassium, 500ppm copper, 1,500 ppm zinc, 180,000 IU/lb vitamin A, 55,000 IU/lb vitamin D_3 and 500 IU/lb vitamin E. Other ingredients on the label (not quantified) include: iron, zinc, magnesium, manganese, choline, cobalt, ascorbic acid, niacin, riboflavin, pyridoxine HCl, pantothenic acid, biotin, vitamin K, vitamin B_{12}, folic acid, carotene and iodine.

Mycotoxin analyses (Midwest Laboratories Inc, Omaha, Nebraska, US) showed 2.08 ppb total aflatoxins and 3.0 ppm total fumonisins in a pooled sample of the GM feed and no aflatoxins and 1.2 ppm total fumonisins in a pooled sample of the non-GM feed. No other mycotoxins were detected. These levels are well below the USA and EU limits for mycotoxins in pig feed. In addition, according to common industry practice, a mycotoxin binding agent (200 mesh bentonite clay) was added to the diets of young pigs (Table 1).

Animals

Standard commercial Yorkshire-cross piglets were obtained from a commercial farrowing facility as a result of crossing Hampshire Duroc males with Yorkshire Landrace females. All sows were fed the same diet containing some GM ingredients and were impregnated at a similar time to obtain isowean piglets. Male piglets were neutered at three days of age in order to fulfill market requirements for meat free of boar-taint.

Unweaned piglets (N=168; average 24 days of age) were transported to the piggery nursery and randomly placed into pens of 14 each. Pens were then randomly allocated to receive either a GM or non-GM diet. Animals were weighed and then fed their allocated diet as their first solid food. After 32 days, pigs were transported to a different facility for the ‘growing and finishing’ phase, where they continued on their allocated diets but were housed as 42 pigs per pen with outside access. Throughout, pigs were housed according

to usual industry practices, under shelter on concrete floors. They experienced the natural daytime/night-time temperature and light/dark cycle.

Data collected from live pigs

Individual weights were recorded weekly and animals were monitored daily by observers who were blinded to a pig's dietary group. Daily measurements included inside and outside air temperature, air quality, weather conditions, level of activity of pigs around the feeder and the appearance of the feeder itself, the level of activity of the pigs around the water and the appearance of the water, details of any pigs found dead, details of any pigs that were moved away from, or back to, the 'home pen' and the reasons for this (e.g. they were being harassed by other pigs), level of contentment (measured as content, irritable or aggressive), presence of cough or sneeze, the presence of any skin problems (e.g. pale or discoloured skin or the presence of rashes or sores), any eye problems, and the consistency of the stools (normal, some loose or runny stools, lots of loose or runny stools). Blood was taken from the jugular vein of awake pigs according to standard industry methods two days before the first pigs were slaughtered. The blood was taken from a random subset of pigs in the following pattern to prevent any time-related bias: approx. half the pigs in the non-GM-fed group, approx half the pigs in GM-fed group, the remainder of the non-GM-fed group, and the remainder of the GM-fed group. Blood was centrifuged and serum was removed and frozen. Blood biochemical analyses were undertaken by Marshfield Clinic Laboratories, Marshfield, WI, USA, who were blinded to all aspects of the study. The laboratory's reference range for awake three to four month-old Yorkshire cross pigs was used as it was most applicable for this study.

Autopsy procedure

When the pigs were 26 weeks old, they were fasted for 18 hours and transported to a large commercial abattoir where they were slaughtered according to the usual, approved methods of the abattoir on two consecutive days. On each day, approximately equal numbers of GM-fed and non-GM-fed pigs were slaughtered to prevent any temporal between-group bias. Pigs on each day were killed within a few minutes of each other. The internal organs were carefully removed to prevent faecal contamination and placed in individual identified buckets with 2 litres of cold phosphate-buffered saline to quickly chill the organs. Organs were kept under near-freezing conditions until they were examined by two licenced, practicing veterinarians with considerable porcine experience. They were blinded as to which pigs were fed GM feed. To remove any between-inspector bias, one veterinarian examined all the kidneys, hearts, lungs and stomachs while the other examined all the livers, spleens, intestines, uteri and ovaries. Veterinarian comments and organ weights were recorded by the same person to remove any between-person measurement bias or recording bias. Where evisceration resulted in incomplete removal of an organ, veterinarians determined if disease had caused part of an organ to adhere to the chest or abdominal wall and hence remain with the carcass, as well as the nature of that disease. The weights of partial organs were not included in statistical analyses due to the errors they would have produced. Kidney weights were the sum of both kidneys per pig. Ovary weights were the sum of both ovaries per pig except for two GM-fed pigs where one ovary was accidentally removed by the abattoir. Here, the weight of both ovaries was estimated by doubling the weight of the remaining ovary. Intestines could not be weighed or inspected due to the amount of digesta still present in them, even after 18 hours of fasting, so the external surface of the intestines was examined for abnormalities

and any intramural, palpable tissue masses. Organ weights were analysed as a percentage of body weights.

In addition to externally examining the organs, veterinarians also examined the interior of every kidney using a single, deep transverse cut, every heart by slicing into both ventricles and both atria, and every lung using at least two deep cuts through the dorsal surface of each lung lobe, and if abnormalities were found, several more cuts to properly identify the abnormality and its extent. Every stomach was examined by cutting it open along the length of its greatest curvature, washing out the contents and inspecting the entire internal surface of the opened-flat stomach, including rugae.

Data analysis

A stomach erosion was defined as an abnormal stomach surface that had a visible area of current inflammation and oedema and where the mucosa was starting to separate (and which could potentially progress to form an ulcer). The length of any ulcer was measured in millimetres. If an ulcer had a clot in it, or showed frank bleeding, it was recorded as a bleeding ulcer. If an ulcer was less than 1 mm in length, it was recorded as a pin-point ulcer, otherwise as a frank ulcer. When calculating the total length of ulceration in each stomach in mm, each pin-point ulcer was numerically rounded to be 1mm in length. Stomach inflammation was scored by the attending, blinded veterinarian as a result of expertise obtained from numerous pig autopsies and a classification system developed as a result of an earlier, preliminary study on pig stomachs. These stomachs were obtained from a random sample of pigs from the same abattoir and came from pigs raised by other commercial pig producers. Inflammation was classified as nil, mild, moderate, or severe based on a combination of the area of current inflammation and level of redness and swelling. Typical examples of each of the four categories of inflammation are shown in Figure 1. For a severe level of inflammation, almost the whole fundus had to be swollen and cherry-red in colour.

Data were analysed using the statistical packages SPSS and EpiInfo. Continuous data were analysed by removing SPSS-identified extreme outliers, being those more than three times the interquartile range away from the lower or upper quartiles. This conservative and well-established approach better tests the nature of the underlying distribution. Data were then tested for normal distribution using the Shapiro-Wilk test. If a normal distribution was found for both dietary groups, data were expressed as means and standard deviations and were analysed using parametric methods (t-test), otherwise data were expressed as medians and ranges and analysed using non-parametric methods (Mann-Whitney U test). Categorical data were analysed using uncorrected chi-squared tests unless an expected cell value was less than five, when Fisher's Exact was used.

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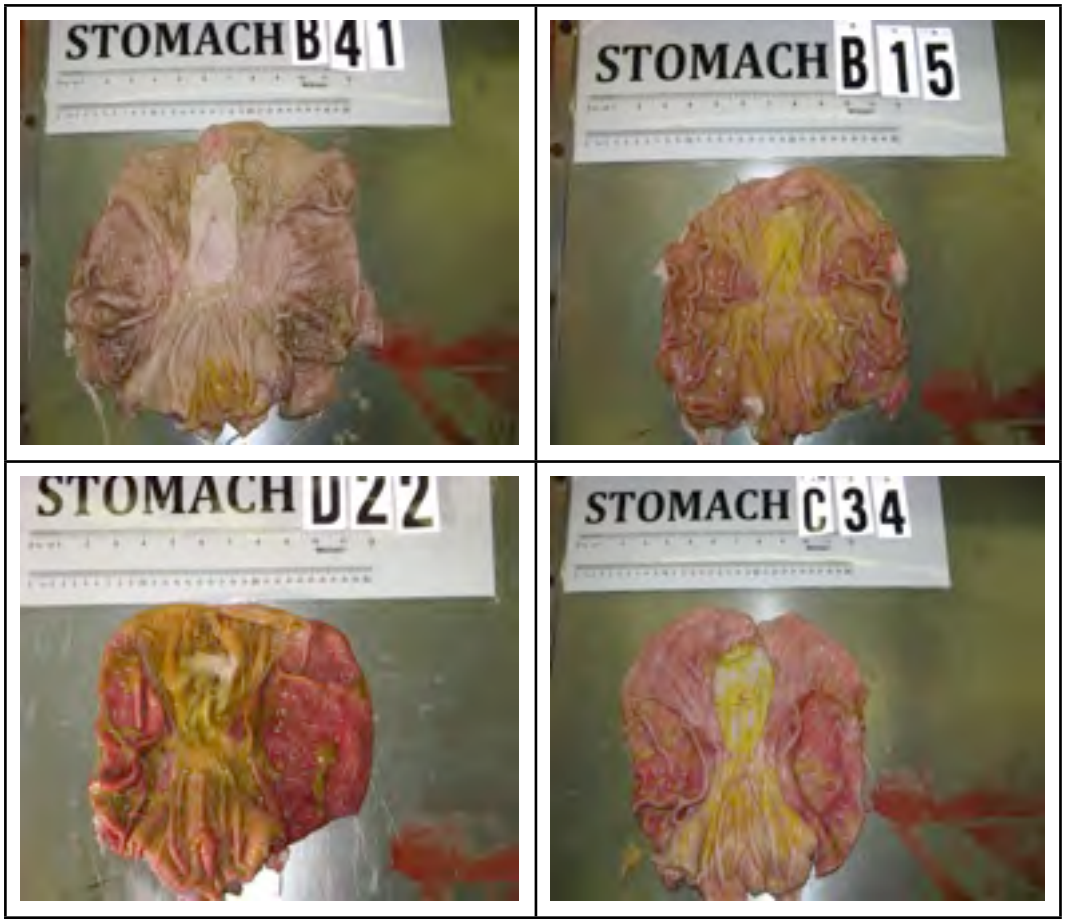

Figure 1. Different levels of stomach inflammation found (clockwise from top left): nil (from a non-GM-fed pig, number B41), mild (from a non-GM-fed pig, number B15), moderate (from a GM-fed pig, number C34) and severe (from a GM-fed pig, number D22).

Results

There were no statistically significant differences in food intake, feed conversion ratios, number or nature of illnesses, number or nature of veterinary interventions, veterinary costs or mortality between the non-GM-fed and GM-fed groups of pigs. Mortalities were 13% and 14% for the non-GM-fed and GM-fed groups respectively, which are within expected rates for US commercial piggeries. All dead pigs were autopsied by blinded veterinarians and deaths were assessed as due to usual commercial piggery-related matters and not to their diets. There was also no difference in body weights between the two dietary groups, initially, during, or at the end of the experiment. Initial weights in kg were : non-GM-fed group: 6.71 + 1.05 (mean + standard deviation); GM-fed group: 6.87 + 0.97. Final weights were: non-GM-fed group: 100.42 + 22.84; GM-fed group: 101.75 + 21.92.

Autopsy results

Organ weights were not statistically different between GM-and non-GM-fed pigs except for uterine weights (Table 2). After removing one extreme outlier, the medians of the non-GM-fed (now N=33) and GM-fed (N=37) groups became 0.084% and 0.105% of the body weight respectively. That is, the median uterus weight of GM-fed pigs, as a proportion of

body weight, was 25% higher than that of non-GM-fed pigs, which was statistically significant (p=0.025).

There was no difference in the disease status of organs between the two groups of pigs except for the level of inflammation in the stomachs of the pigs (Table 3, Figure 1). For non-GM-fed pigs, stomach inflammation was concentrated in the mild and moderate range, whereas GM-fed pigs showed much more severe inflammation (p=0.004). GM-fed pigs showed severe stomach inflammation at a rate of 2.6 times that of non-GM-fed pigs (95% confidence interval = 1.29-5.21) (Table 3). This occurred in both male (p=0.041) and female (p=0.034) pigs (Table 4). We found severe stomach inflammation in 22.2% of male pigs fed the GM diet and in 41.7% of female pigs fed the GM diet (compared to 5.6% and 18.9%, respectively, in pigs fed the non-GM diet (Table 4).

Blood biochemistry

Blood biochemistry results are given in Table 5. Aspartate transaminase (AST), potassium and creatine kinase (CK) were not statistically analysed because they were raised substantially in both dietary groups due to the way blood was collected and hence they were unable to reflect any effect of feeding a GM diet. AST and potassium were raised because the collection needle was pushed through muscle, while CK was raised due to the pigs being alert and restrained while blood was taken. While bicarbonate can increase if pigs pant or squeal unduly during blood taking, no pigs recorded a bicarbonate concentration higher than the reference range (Table 6), so this variable was retained in analyses.

To determine if feeding the GM diet was associated with a clinically abnormal biochemistry profile, the proportion of pigs in each dietary group that lay above (or below) the reference (normal) range were then compared (Table 6). No statistically significant differences were found. The means or medians of the biochemical variables were also compared. No significant differences were found (Table 5).

The analyses of several biochemical variables were confounded by the level of haemolysis in the blood sample. Haemolysis can be a problem when taking blood from alert animals, and in non-laboratory settings due to lag times between sampling and centrifuging blood. Haemolysis was reported as nil, mild, moderate or severe by the laboratory. Total bilirubin, urea nitrogen, creatinine, phosphorus, calcium, sodium, chloride, bicarbonate, and anion gap were found to be significantly correlated with the level of haemolysis (results not shown) and hence haemolysis was regarded as a confounder for these variables. Spearman's rho test was used as a measure of the association rather than the Pearson correlation co-efficient as it is less sensitive to outliers and does not assume normality. These biochemical variables then underwent multiple linear regression to control for the effect of haemolysis. As known confounders should be controlled-for, even if they do not appear as actual confounders in initial studies, glucose also underwent this process. No biochemical variable was found to have a significant relationship to the diet with the level of haemolysis controlled-for (results not shown). Consequently, no biochemical differences were found between non-GM-fed and GM-fed pigs. However, the concentration of GGT, which is a measure of liver heath, was 16% lower in GM-fed pigs than non-GM-fed pigs and this result was on the borderline of statistical significance (Table 5).

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Table 2. Organ weights (as a percentage of body weight) - descriptive statistics of raw data and statistical comparisons of extreme outlier-removed data.

	Non-GM-fed						GM-fed						Statistical comparison of dietary groups	
	n[a]	Mean	SD[b]	Median	Min	Max	n[a]	Mean	SD[b]	Median	Min	Max	Test used[c]	p[d]
Kidneys	66	0.32	0.066	0.31	0.19	0.66	68	0.33	0.057	0.32	0.16	0.56	t	0.51
Heart	69	0.40	0.065	0.40	0.27	0.63	69	0.41	0.059	0.40	0.27	0.61	MW	0.79
Liver	71	1.81	0.342	1.77	1.27	3.20	72	1.79	0.348	1.71	1.25	3.16	MW	0.45
Spleen	73	0.16	0.033	0.16	0.11	0.33	71	0.16	0.032	0.15	0.093	0.30	t	0.40
Lung	67	0.91	0.241	0.87	0.58	2.00	68	0.98	0.315	0.94	0.57	2.52	MW	0.20
Stomach	73	0.62	0.130	0.57	0.42	0.99	71	0.64	0.129	0.60	0.44	1.01	MW	0.26
Uterus	34	0.10	0.048	0.086	0.040	0.31	37	0.12	0.053	0.105	0.036	0.244	MW	0.025*
Ovaries	36	0.0085	0.0027	0.0081	0.0040	0.019	36	0.0086	0.0023	0.0084	0.0047	0.014	t	0.38

a An organ was not included in the analysis if adhesions caused only a partial organ to remain with the viscera, due to the errors inclusion would have caused.
b Standard deviation
c After tests for normality, groups were compared by 2-tailed t-test if data from both dietary groups were normally distributed, Mann Whitney U test (MW) otherwise.
d* p<0.05 to 0.01, ** p<0.01 to 0.001, *** p<0.001

Table 3. The proportion of pigs in each dietary group with adverse findings on gross pathology

Organ	Condition	Proportion with condition				Relative risk of condition in GM-fed pigs	95% confidence interval of the relative risk	p[a]
		Non-GM-fed		GM-fed				
		No. N=73	%	No. N=72	%			
Kidney	Any abnormality	0	0.0	0	0.0	—[b]	—[b]	—[b]
Heart	Any abnormality[c]	11	15.1	5	6.9	0.46	0.17-1.26	0.119
Liver	Any abnormality[d]	6	8.2	3	4.2	0.51	0.13-1.95	0.494
Spleen	Any abnormality[e]	3	4.1	2	2.8	0.68	0.12-3.93	1.000
Lung	Pneumonia[f]	42	57.5	43	59.7	1.04	0.79-1.36	0.789
	Fibrous pleuritis or pericarditis	9	12.3	4	5.6	0.45	0.15-1.40	0.153
	Abnormal lymph nodes[g]	13	17.8	16	22.2	1.25	0.65-2.40	0.506
Stomach	Nil inflammation	4	5.4	8	11.1	2.03	0.64-6.44	0.218
	Mild inflammation	31	42.5	23	31.9	0.75	0.49-1.16	0.190
	Moderate inflammation	29	39.7	18	25.0	0.63	0.39-1.03	0.058
	Severe inflammation	9	12.3	23	31.9	2.59	1.29-5.21	0.004**
	Erosion(s)	63	86.3	58	80.6	0.93	0.81-1.08	0.352
	Pin-point ulcer(s)	13	17.8	9	12.5	0.70	0.32-1.54	0.373
	Frank ulcer(s)	15	20.5	17	23.6	1.15	0.62-2.12	0.657
	Bleeding ulcer(s)	0	0.0	2	2.8	—[b]	—[b]	0.245
Intestines	Any abnormality	0	0.0	0	0.0	—[b]	—[b]	—[b]
Uterus	Filled with fluid[h]	0[i]	0.0	2[j]	5.6	—[b]	—[b]	0.493
Ovary	Any abnormality	0[k]	0.0	0[l]	0.0	—[b]	—[b]	—[b]

a Uncorrected chi-square test unless an expected cell value was less than five, when Fisher exact test (2-tailed) was used. * p<0.05 to 0.01, ** p<0.01 to 0.001, *** p<0.001
b No statistic could be calculated because one or more cells contained zeros.
c Adhesions and/or fibrous pericarditis and/or scar tissue.
d Adhesions and/or fibrinous tags and/or the presence of fibrin.
e Adhesions and/or fibrinous tags.
f Consolidating bronchopneumonia of the cranial ventral lung lobe(s) and/or caudal lobe(s).
g Haemorrhagic and/or swollen bronchial lymph node(s).
h When two uteri were removed from neighbouring organs, fluid oozed from them.
i N=36. Of 37 females, one had a congenital defect. It had only the beginnings of a uterine tract and no uterus or ovaries.
j N=36.
k N=36. Of 37 females, one had a congenital defect. It had only the beginnings of a uterine tract and no uterus or ovaries.
l N=35. Of 36 females, one had a uterus but no ovaries, which were removed by accident during slaughter and retained by the slaughterhouse.

Table 4. Stomach inflammation by gender.

Gender	Level of stomach inflammation	Proportion with condition				Relative risk of condition in GM-fed pigs	95% confidence interval of the relative risk	p[a]
		Non-GM-fed		GM-fed				
		No.[b]	%	No.[c]	%			
Males	Nil	1	2.8	4	11.1	4.00	0.47-34.07	0.357
	Mild	16	44.4	12	33.3	0.75	0.42-1.35	0.334
	Moderate	17	47.2	12	33.3	0.71	0.40-1.26	0.230
	Severe	2	5.6	8	22.2	4.00	0.91-17.56	0.041*
Females	Nil	3	8.1	4	11.1	1.37	0.33-5.70	0.711
	Mild	15	40.5	11	30.6	0.75	0.40-1.41	0.373
	Moderate	12	32.4	6	16.7	0.51	0.22-1.22	0.118
	Severe	7	18.9	15	41.7	2.20	1.02-4.76	0.034*

a Uncorrected chi-square test unless an expected cell value was less than five, when Fisher exact test (2-tailed) was used. * p<0.05 to 0.01, ** p<0.01 to 0.001, *** p<0.001
b N=36 for males, N=37 for females.
c N=36 for males, N=36 for females.

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Table 5. Blood biochemistry descriptive statistics of raw data and statistical comparisons of extreme outlier-removed data.

	Non-GM-fed			GM-fed			Reference range[a]		Statistical comparison of dietary groups	
	N	Median[b] (Mean)	Range[b] (SD)	N	Median[b] (Mean)	Range[b] (SD)	Standard (asleep)[c]	Awake (Yorkshire X)[d]	Test used[e]	p[f]
Glucose (mg/dL)	39	89.0	58 – 109	38	90.5	52 – 111	85 – 150	58.0 – 197.0	MW	0.81
AST[g](U/L)	39	60.0	21 – 2757	38	57.0	12 – 1724	32 – 84	0.0 – 45.0	MW	0.72
Total bilirubin (mg/dL)	39	0.10	0.1 – 0.3	38	0.10	0.1 – 0.3	0.0 – 1.0	0.1 – 0.2	MW	0.76
Cholesterol (mg/dL)	39	100.0	56 – 140	38	100.0	55 – 125	36 – 54	50.0 – 92.0	MW	0.85
Total protein (g/dL)	39	(6.48)	(0.95)	38	(6.63)	(0.91)	7.9 – 8.9	5.1 – 6.9	t	0.16
Albumin (g/dL)	39	4.00	1.7 – 4.7	38	4.10	1.7 – 4.8	1.9 – 3.3	3.0 – 4.4	MW	0.59
Urea nitrogen (mg/dL)	39	11.0	5 – 22	38	12.0	8 – 29	10 – 30	4.3 – 12.7	MW	0.30
Creatinine (mg/dL)	39	0.90	0 – 1	38	0.70	0 – 1	1.0 – 2.7	0.9 – 1.9	MW	0.21
Phosphorus (mg/dL)	39	(9.1)	(1.5)	38	(9.1)	(1.5)	5.3 – 9.6	6.2 – 9.2	t	0.99
Calcium (mg/dL)	39	10.70	5.5 – 11.3	38	10.50	5.1 –12.0	7.1 –11.6	9.1 – 10.8	MW	0.94
Sodium (mmol/L)	37	140.0	98 – 148	37	140.0	98 – 145	135 - 150	132.0–144.0	MW	0.60
Potassium (mmol/L)	38	6.35	4.6 – 13.9	37	6.40	4.3 –16.3	4.4 – 6.7	3.4 – 5.0	MW	0.56
Chloride (mmol/L)	38	97.0	67 – 104	37	98.0	66 – 102	94 – 106	94.0 – 103.0	MW	0.86
Bicarbonate (mmol/L)	39	33.0	19 – 37	38	33.5	18 – 37	18 – 27	28.0 – 37.0	MW	0.44
CK[h] (U/L)	39	2416.0	214 –22500	38	1960.0	10 –22500	61 –1251	264.0–1247.0	MW	0.73
GGT[i](U/L)	39	(35.1)	(18.4)	38	(29.5)	(18.1)	10 – 60	0.0 – 60.0	t	0.05
Anion gap (mmol/L)[j]	37	16.0	12 – 23	37	15.0	11 – 27	–	–	MW	0.61

a From Marshfield Clinic, Marshfield, WI, USA.
b Medians and ranges are reported for non-parametric comparisons, means and standard deviations for parametric comparisons.
c Marshfield Clinic's usual reference range. Pigs were anaesthetised to obtain blood.
d Marshfiled Clinic's reference range for awake, 3-4 month-old Yorkshire cross pigs. This was used as it is much more applicable to this study.
e After tests for normality, groups were compared by two-tailed t-test if data from both dietary groups were normally distributed, Mann Whitney U test (MW) otherwise.
f * p<0.05 to 0.01, ** p<0.01 to 0.001, *** p<0.001
g Aspartate transaminase.
h Creatine kinase.
i Gamma-glutamyl transferase.
j There is no laboratory reference range for anion gap. Sorbitol dehydrogenase results were not given by the lab on this occasion.

Table 6. Biochemical variables compared to the reference range[a] to determine clinical significance.

Biochemical variable	Number (%) above or below reference range			
	Non-GM-fed (N=39)		GM-fed (N=38)	
	Above reference range	Below reference range	Above reference range	Below reference range
Glucose	0 (0)	0 (0)	0 (0)	2 (5)
AST[b]	23 (59)	—[c]	24 (63)	—[c]
Total bilirubin	1(3)	0 (0)	1 (3)	0 (0)
Cholesterol	29 (74)	0 (0)	28 (74)	0 (0)
Total protein	10 (26)	4 (10)	17 (45)	3 (8)
Albumin	7 (18)	5 (13)	3 (8)	5 (13)
Urea nitrogen	10 (26)	0 (0)	16 (42)	0 (0)
Creatine	0 (0)	18 (46)	0 (0)	23 (61)
Phosphorus	12 (31)	2 (5)	16 (42)	1 (3)
Calcium	10 (26)	9 (23)	14 (37)	6 (16)
Sodium	2 (5)[d]	4 (11)[d]	0 (0)[d]	4 (11)[d]
Potassium	34 (89)[e]	0 (0)[e]	36 (97)[d]	0 (0)[d]
Chloride	1 (3)[e]	7 (18)[e]	0 (0)[d]	4 (11)[d]
Bicarbonate	0 (0)	5 (13)	0 (0)	5 (13)
CK[f]	24 (62)	2 (5)	27 (71)	1 (3)
GGT[g]	2 (5)	—[c]	1 (3)	—[c]

a Awake Yorkshire cross pig reference range from Marshfield Clinic, Marshfield, WI, USA. Anion gap has no reference range so was not included in the table.
b Aspartate transaminase.
c It was not possible for a pig to record a concentration below the bottom of the reference range, which was zero.
d N=37.
e N=38.
f Creatine kinase.
g Gamma-glutamyl transferase.

Discussion

In this study, we found that female pigs fed the GM diet had median uterine weights that were 25% greater than non-GM-fed pigs (p=0.025). This result is attributed to the difference in diet as other variables were controlled for, including the presence of mycotoxins, and possible confounders such as infectious diseases, animal husbandry considerations and various forms of bias such as temporal, between-person, measurement or recording bias, as these were all controlled-for. The concentration of mycotoxins in the feed was insignificant, both dietary groups received the same nutrients and care, the care complied with industry standards, and all those doing laboratory analyses and weighing, caring for, slaughtering and doing autopsies on pigs were blinded as to the dietary group of each pig.

The reported difference in uterine weight warrants further investigation in future studies because such a biologically significant difference in uterine weights may reflect endometrial hyperplasia or carcinoma, endometritis, endometriosis, adenomyosis, inflammation, a thickening of the myometrium, or the presence of polyps. The uteri from two GM-fed pigs were full of fluid compared to nil from non-GM-fed pigs (Table 3) which may be linked to pathology. The link between an increase in uterine weights and GM feeding is supported by other authors (Brasil et al., 2009) who found that GM soy-fed rats had a statistically significant 59% increase in the density of the uterine endometrial glandular epithelium compared to rats fed an equivalent organic soy diet. Further studies should include histology, blood oestrogen, progesterone and cytokine concentrations, and which GM crop(s) and their GM protein products may, or may not, be involved. As this study used neutered males, further studies are required to investigate any potential effect of these crops on male reproduction. Multigenerational reproductive studies should also be considered.

In this study, a diet of GM feed had no effect on stomach erosions or ulceration but had a significant effect on inflammation. Pigs fed the mixed GM soy and GM corn diet showed 2.6 times the rate of severe stomach inflammation compared to non-GM fed pigs. This biologically significant finding was statistically significant ($p=0.004$). GM-fed male pigs showed severe stomach inflammation at a rate of 4.0 times that of the non GM fed male pigs ($p=0.041$); and female pigs showed a rate of severe stomach inflammation that was 2.2 the rate of the non-GM fed female pigs ($p=0.034$).

The pig industry uses finely-ground feed to maximise feed efficiency which can increase inflammation and ulceration of the stomach (Wolf, 2010). We therefore controlled the grind size, removing it as a confounder. Hence our results show that these GM crops were associated with stomach inflammation that was additional to any that may be caused by particle size. The result is attributed to the difference in diet, since the presence of mycotoxins, possible confounders such as infectious diseases, animal husbandry considerations or temporal, between-person, measurement and recording bias were controlled across the two groups.

One explanation for the inflammation results could lie with the Cry 3Bb1 and Cry 1Ab proteins that these GM corn varieties are engineered to produce. They act as insecticides by inducing pore formation and disintegration of the gut tissue (Spok et al., 2007) of certain grubs that attack corn plants. It has been argued that these proteins cannot harm the gastrointestinal tract of mammals because mammals lack the necessary gut environment and receptors (ANZFA, 2000). However, Vazquez-Padron et al. (2000) found six proteins in the mouse small intestine that could bind to a Cry protein (Cry 1Ac). Furthermore, when the Cry protein bound to these proteins, it resulted in hyperpolarisation of the intestine, which is consistent with the formation of cationic channels, as occurs in the insect gut (Vazquez-Padron et al., 2000). In addition, an independent in vivo study found structural changes and hyperplasia in the ileum of mice fed a Cry protein for two weeks (Fares & El-Sayed, 1998). Chowdhury et al. (2003) and Walsh et al. (2012b) found the Cry1Ab protein (which was present in the feed in our study) throughout the digestive tract of pigs. Chowdhury et al. (2003) found the protein (and sections of the gene that codes for it) in the stomach, duodenum, ileum, caecum and rectum of pigs fed Bt11 corn for four weeks, while Walsh et al. (2012b) found the protein in the stomach, caecum and colon of pigs fed MON810 corn for 110 days (they

appear not to have looked in the rectum), indicating that this protein is resistant to digestion in pigs. In our study, stomach inflammation may be due to one or both of the Cry proteins fed in the study and future studies may provide answers.

The findings in this study are conservative since the non-GM diet pigs were exposed, albeit minimally, to potential GMO impacts. The presence of small amounts of GM material in the non-GM feed, using out-bred animals, piglets from GM-fed sows, and performing the study in a commercial setting (including the potential exposure of the pigs to any infectious diseases common to US commercial pigs and taking blood on site) could be expected to reduce any differences between the two dietary groups.

We found that our key findings were not reflected in the standard biochemical tests often undertaken by researchers in this area, probably because such tests provide a poor measure of inflammation and matters associated with uterine size. We suggest that the following may be better measures: the red blood cell count and haematocrit to measure anaemia and iron deficiency from possible blood loss, C-reactive protein and white blood cell count to measure inflammation, and oestrogen and progesterone.

In addition, if an autopsy is done at the end of a GM crop feeding experiment, this often involves only a visual inspection of the exterior of organs without weighing them. However by weighing organs we found a significant 25% increase in uterine weights in the GM-fed pigs. Moreover, where organs are weighed in such studies, they are often not examined internally (Carman, 2004) and such an approach would preclude finding the stomach inflammation reported in the present study.

The present study is an observational study of the action of a mixture of GM crops on the health of pigs, versus a comparable non-GM diet. Future work will investigate individual GM crops, will involve histopathology, and will consider mechanisms for reported group differences.

Conclusion

Pigs fed a GMO diet exhibited heavier uteri and a higher rate of severe stomach inflammation than pigs fed a comparable non-GMO diet. Given the widespread use of GMO feed for livestock as well as humans this is a cause for concern. The results indicate that it would be prudent for GM crops that are destined for human food and animal feed, including stacked GM crops, to undergo long-term animal feeding studies preferably before commercial planting, particularly for toxicological and reproductive effects. Humans have a similar gastrointestinal tract to pigs, and these GM crops are widely consumed by people, particularly in the USA, so it would be be prudent to determine if the findings of this study are applicable to humans.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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Review

A literature review on the safety assessment of genetically modified plants

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ABSTRACT

In recent years, there has been a notable concern on the safety of genetically modified (GM) foods/plants, an important and complex area of research, which demands rigorous standards. Diverse groups including consumers and environmental Non Governmental Organizations (NGO) have suggested that all GM foods/plants should be subjected to long-term animal feeding studies before approval for human consumption. In 2000 and 2006, we reviewed the information published in international scientific journals, noting that the number of references concerning human and animal toxicological/health risks studies on GM foods/plants was very limited. The main goal of the present review was to assess the current state-of-the-art regarding the potential adverse effects/safety assessment of GM plants for human consumption. The number of citations found in databases (PubMed and Scopus) has dramatically increased since 2006. However, new information on products such as potatoes, cucumber, peas or tomatoes, among others was not available. Corn/maize, rice, and soybeans were included in the present review. An equilibrium in the number research groups suggesting, on the basis of their studies, that a number of varieties of GM products (mainly maize and soybeans) are as safe and nutritious as the respective conventional non-GM plant, and those raising still serious concerns, was currently observed. Nevertheless, it should be noted that most of these studies have been conducted by biotechnology companies responsible of commercializing these GM plants. These findings suggest a notable advance in comparison with the lack of studies published in recent years in scientific journals by those companies. All this recent information is herein critically reviewed.

Contents

1. Introduction

In recent years, the use and release of genetically modified organisms (GMOs) has been an issue of intense public concern and, in the case of foods, products containing GMOs or products thereof carry the risk of consumer rejection. The World Health Organization (WHO) defines GMOs as those organisms in which the genetic material has been altered in a way that does not occur naturally (WHO, 2002). As genetically modified (GM) foods are starting to be present in our diet concerns have been expressed regarding GM food safety (Dona and Arvanitoyannis, 2009). Although the WHO declares that the GM products that are currently on the international market have all gone through risk assessment by national authorities, the risk assessment of GM foods in general, and crops in particular for human nutrition and health, has not been systematically performed as indicated in the scientific literature (Domingo, 2007; Magaña-Gómez and de la Barca, 2009). Evaluations for each GM crop or trait have been conducted using different feeding periods, animal models, and parameters. The most common result is that GM and conventional sources induce similar nutritional performance and growth in animals. However, adverse microscopic and molecular effects of some GM foods in different organs or tissues have been reported to a

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certain extent (Magaña-Gómez and de la Barca, 2009). Diversity among the methods and results of the risk assessments reflects the complexity of the subject.

Among the different GMOs, in recent years GM plants have attracted a large amount of media attention. However, the general public remains largely unaware of the real notion of GM plants or what advantages and disadvantages the technology has to offer, particularly with regard to the range of applications for which they can be used. From the first generation of GM crops, two main areas of concern have emerged, namely risk to the environment and risk to human health. As GM plants are gradually being introduced into the European Union it is likely that public concern regarding potential health issues will arise. Although it is now commonplace for the press and media to adopt 'health campaigns', the information they publish is often unreliable and unrepresentative of the available scientific evidence (Key et al., 2008).

Approximately 15 years have passed after the introduction of genetic modifications in food, and new GM products are currently added to the existing list of foods. However, 10 years ago we already noticed that there was no sufficient published information concerning safety of GM foods in general, and GM plants, in particular. Specifically, the lack of published toxicological studies on adverse health effects was evident (Domingo, 2000; Domingo-Roig and Gómez-Arnáiz, 2000). In 2006, 6 years after our initial review was published, we carried out a new review of the scientific literature on the potential adverse health/toxic effects of GM/transgenic plants (Domingo, 2007). Studies about the safety of the potential use of potatoes, corn, soybeans, rice, cucumber, tomatoes, sweet pepper, peas, and canola plants for food and feed were included in that review. The number of references found in the databases was yet surprisingly limited. Moreover, most published studies were not performed by the biotechnology companies that produce/commercialize these products. However, as it also occurred with our first review (Domingo, 2000), we found a considerable number of references concerning commentaries, general news, and letters to the Editor (published in reputable international journals). Notwithstanding, papers about experimental investigations on the safety of GM foods/plants were very scant. Hence, the conclusion from our 2006 review (Domingo, 2007) was, for the second time, that if data on toxicological assessment of GM foods/plants existed, these had not been reported in scientific journals, and therefore, they were not available to the general scientific judgment.

Probably, one of the most important problems related with the lack of studies (at least not published in the scientific literature) on the safety assessment of GM foods/plants was the use of the "substantial equivalence" concept. This notion is based on the principle: "if a new food is found to be substantially equivalent in composition and nutritional characteristics to an existing food, it can be regarded as being as safe as the conventional food" (SOT, 2003). Although application of the concept is not a safety assessment per se, it enables the identification of potential differences between the existing food and the new product, which should then be further investigated with respect to their toxicological impact. Why must it be thought that two plants (GM and non-GM) with the same nutritional capacity should also imply similar health risks (or absence of risks)? Why a similar principle is not used, for example, for chemical substances of commercial interest such as pesticides, drugs, food additives, etc.? In fact, the "substantial equivalence" principle is a starting point rather than an end point (Kuiper et al., 2002). If this seems to be reasonably obvious, and taking into account the great controversy generated by the debate about GM plants safety, why the published information is so scarce?

The conclusions of our 2006 review concerning the doubts on the use of the principle of "substantial equivalence" in GM plants, as well as the lack of toxicological studies (Domingo, 2007), were quite in agreement with the conclusions of other reviews (Zduńczyk, 2001; Bakshi, 2003; Pryme and Lembcke, 2003), as well as with those of our previous review (Domingo, 2000; Domingo-Roig and Gómez-Arnáiz, 2000). In a recent paper (Dona and Arvanitoyannis, 2009), it was reported that the results of most studies with GM foods indicated that they might cause some common toxic effects. There is no doubt that one of the main issues concerning GM food safety assessment is based upon detection of their potentially toxic properties, which could provoke unintended effects of the genetic modification (Tyshko et al., 2007).

2. Risk assessment of GM plants

In our previous two reviews (Domingo, 2000, 2007), as well as in the current one, the scientific literature on the potential adverse health/toxic effects of GM/transgenic foods/plants was reviewed using the PubMed database (available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed). In our first review, the search covered the period January 1980–May 2000, while the second review covered the period January 1980–October 2006. The current one covers the period January 1980–August 2010. We initially used the following "key terms": genetically modified foods, GM foods, transgenic foods, toxicity of transgenic foods, health risks of transgenic foods, adverse effects of genetically modified foods, toxicity of genetically modified foods, health risks of GM foods, health risks of genetically modified foods, toxicity of GM foods, adverse effects of GM foods, and adverse effects of transgenic foods. Citations corresponding to general "key terms" such as: genetically modified foods, GM foods, and transgenic foods were, not surprisingly, quantitatively the most important. After this preliminary screening, our search was focused in these four terms: (a) genetically modified foods, (b) toxicity of transgenic foods, (c) adverse effects of transgenic foods, and (d) health risks of transgenic foods. The number of citations has dramatically grown in recent years. Thus, in 2000, 2006 and 2010, those numbers were respectively: 101, 686 and 2879 for (a); 44, 136 and 376 for (b); 67, 199 and 504 for (c), and 3, 23 and 75 for (d) (Fig. 1). In spite of the notable increase in the number of citations, those concerning specifically to studies focused on demonstrating the health safety of GM foods remain very limited. Given that mentioned earlier, it is noteworthy that search terms such as "substantial equivalence" were not considered herein aiming to avoid any misleading information on the possible toxicological/safety concerns of GM crops to human health.

The present review, as our previous one (Domingo, 2007), was focused on GM plants only, a group of GMOs for which an especial interest exists for their potential use in food and feed. In addition to PubMed (Pub), we have also used Scopus (Sc) as database for the present online search. The number of references found between January 1980 and August 2010 were the following: for toxicity of genetically modified plants, 508 (Pub) and 339 (Sc), for adverse effects of genetically modified plants, 702 (Pub) and 156 (Sc), and for health risks of genetically modified plants, 168 (Pub) and 321 (Sc) (Fig. 1). Comparing the citations related to genetically modified potatoes, cucumber, tomatoes, sweet pepper, peas and canola, with those corresponding to the same products in our previous review (Domingo, 2007), it must be noted that no new toxicological/adverse effects/health risks studies references are available. In contrast, new information (October 2006–August 2010) was found concerning corn, soybean and rice, which is next reported.

2.1. Corn/maize

In the last few years, one of the most active research groups focusing its investigations on GM maize is that of Dr. Séralini and coworkers from the University of Caen (Caen, France). These authors reanalyzed data from a 90-day toxicity study performed in rats under the responsibility of Monsanto Company with a transgenic corn MON

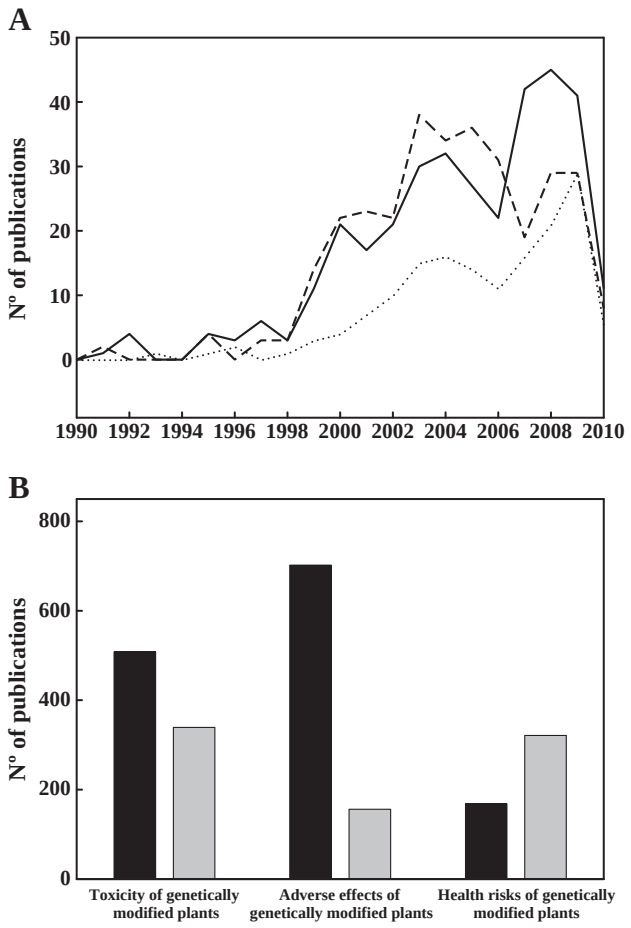

Fig. 1. (A) Number of publications per year, from 1990 to present, referring to (—) toxicity of genetically modified plants, (···) adverse effects of genetically modified plants and (- -) health risks of genetically modified plants, using the Scopus database. (B) Comparison between total number of publications using different keywords with Scopus (▒) and PubMed (■) databases.

863 (a genetically engineered corn variety that contains the gene for modified *Bacillus thuringiensis* (Bt) Cry3Bb1 protein to protect against corn rootworm). MON 863 had been subjected to questions from regulatory reviewers in Europe, where it was finally approved in 2005. Séralini et al. (2007) reported that after the consumption of MON 863, animals showed slight but dose-related significant variations in growth for both sexes, resulting in 3.3% decrease in weight for males and 3.7% increase for females. Moreover, signs of hepatorenal toxicity, marked also by differential sensitivities in males and females, were also noticed, while triglycerides increased by 24–40% in females (either at week 14, dose 11% or at week 5, dose 33%, respectively). In turn, urine phosphorus and sodium excretions diminished in males by 31–35% (week 14, dose 33%), being the most important results significantly linked to the treatment in comparison to seven diets tested. It was concluded that longer experiments were essential in order to indicate the real nature and extent of the possible pathology. It was remarked that based on the Monsanto data, it could not be concluded that GM corn MON 863 was a safe product (Séralini et al., 2007).

An Expert Panel (Doull et al., 2007) was subsequently convened to assess the original study results as analyzed by the Monsanto Company, and the reanalysis conducted by Séralini's group. The Expert Panel concluded that the reanalysis conducted by Séralini et al. (2007) provided no evidence to indicate that MON 863 was associated with adverse effects in the 90-day rat study. In each case, statistical findings reported by both Monsanto and Séralini et al. (2007) were considered to be unrelated to treatment or of no biological or clinical importance because they failed to demonstrate a dose–response relationship, reproducibility over time, association with other relevant changes (e.g., histopathology), occurrence in both sexes, difference outside the normal range of variation, or biological plausibility with respect to cause-and-effect. In a recent review (Séralini et al., 2009), the authors assumed that the methodology used in their previous paper (Séralini et al., 2007) was appropriate to discriminate potential false positive and GM-linked effects, avoiding to some extent false negative results, in the best manner it may be done for somehow too limited protocols already in use for commercialized GMOs (Séralini et al., 2007). Accordingly, the authors (Séralini et al., 2009) declared that GM-linked effects in the 90 days feeding studies were signs of toxicity rather than proofs of toxicity by itself. Besides, it was pointed out, that the biological plausibility of a subchronic or chronic side effect of the GM diet, either linked to the new toxin in the mammalian regimen or due to the mutagenesis effect of the genetic modification itself, was consequently non negligible (Séralini et al., 2009).

Recently, de Vendômois et al. (2009) performed, for the first time, a comparative analysis of blood and organ system data from trials with rats fed three main commercialized GM maize (NK 603, MON 810 and MON 863). The authors found for the 3 GMOs new side effects linked with GM maize consumption, which were sex- and often dose-dependent. Effects were mostly associated with the kidney and liver, the dietary detoxifying organs, although different between the 3 GMOs. Other effects were also observed in heart, adrenal glands, spleen and hematopoietic system. It was concluded that these data highlighted signs of hepatorenal toxicity, possibly due to the pesticides specific to each GM corn (glyphosate and AMPA in NK 603, modified Cry1Ab in MON 810 and modified Cry3Bb1 in MON 863). In addition, unintended direct or indirect metabolic consequences of the genetic modification could not be excluded. To date, and to the best of our knowledge, this study has not been scientifically questioned. Statistically significant effects of GM diets, or of residues of pesticides containing GMOs, have been also previously observed in some (Malatesta et al., 2002a, 2003; Vecchio et al., 2004), but not in all studies (Brake and Evenson, 2004; Brake et al., 2004) enlightening the necessity of a case-by-case approach and that toxicological studies are quite limited, up to date, for this approach (Domingo, 2007). For the Séralini's group it seems unbelievable that a risk assessment carried out only on forty rats of each sex receiving GM rich diets for 90 days (yielding results often at the limits of significance) has not been repeated and prolonged independently.

With regard to the above, it is important to note that according to a recent report of the EFSA GMO Panel working group on animal feeding trials (EFSA, 2008), the aim of the 90-days rodent feeding study with the whole GM food and feed is mainly focused on assessing potential unintended effects of toxicological and/or nutritional relevance and to establish whether the GM food and feed is as safe and nutritious as its traditional counterpart rather than determining qualitative and quantitative intrinsic toxicity of defined food constituents. A 90-day animal feeding trial has a large capacity (sensitivity and specificity) to detect potential biological/toxicological effects of single well defined compounds (Knudsen and Poulsen, 2007). Therefore, it should be possible to model the sensitivity of the rat subchronic feeding study for the detection of hypothetically increased amount of compounds such as anti-nutrients, toxicants, or secondary metabolites. However, with respect to the detection of potential unintended effects in whole GM food and feed, the EFSA GMO Panel also indicates that it would be unlikely that substances present in small amounts, and with a low toxic potential, could result in any observable (unintended) effects in a 90-day rodent feeding study, as they would be below the no-observed-effect-level (NOEL), and thus of unlikely impact to human health at normal intake levels (EFSA, 2008). It is worthy of being mentioned that the EFSA GMO Panel employs the term “unlikely” a couple of times in a few lines, which may suggest certain potential

limitations in the conclusions of 90-day rodent feeding studies performed with GM food and feed.

In contrast to the concern raised in the studies by Séralini and coworkers, other investigators reported that various GM maize grains were as safe as conventional maize grains. The most active group of researchers supporting this is headed by Dr. Delaney, who has published a notable number of papers on this topic since 2007. The conclusions of these studies are next summarized. MacKenzie et al. (2007) performed a subchronic (approximately 90 days) feeding study in Sprague–Dawley rats fed diets containing 1507 maize grain. Maize line 1507 is a GM maize plant that expresses the cry1F gene from Bt sbsp. aizawai and the phosphinothricin-N-acetyltransferase (pat) gene from *Streptomyces viridochromogenes* throughout the plant including the grain. Expression of the Cry1F protein confers to the plant resistance to the European corn borer and other lepidopteron pests. No significant differences were observed in the nutritional performance variables, clinical and neurobehavioral signs, ophthalmology, clinical pathology (hematology, clinical chemistry, coagulation, and urinalysis), organ weights, and gross and microscopic pathology between any pair of treatment groups. In turn, when compared to control groups, Malley et al. (2007) did not find adverse diet-related differences in rats fed given 59122 maize grain with respect to body weight/gain, food consumption/efficiency, clinical signs of toxicity, mortality, ophthalmology, neurobehavioral (FOB and motor activity) assessments, clinical pathology (hematology, clinical chemistry, coagulation, and urinalysis), and pathology (organ weights and gross and microscopic pathology). 59122 is a transgenic maize line containing event DAS-59122-7 that expresses the corn rootworm (CRW) specific pesticidal Cry34Ab1 and Cry35Ab1 proteins from Bt Berliner strain PS149B1 and the phosphinothricin-N-acetyltransferase (PAT) protein from *Streptomyces viridochromogenes* for tolerance to the herbicidal ingredient glufosinate-ammonium. According to the authors, the results of their studies indicated that 1507 and 59122 maize grains were nutritionally equivalent to and as safe as conventional (non-GM) maize grain (MacKenzie et al., 2007; Malley et al., 2007).

In Sprague–Dawley rats, Appenzeller et al. (2009a) conducted a subchronic feeding study to evaluate the potential health effects of long-term consumption of a rodent diet containing 1507 × 59122 maize grains compared with a diet containing maize grain from its near-isogenic control (091). 1507 × 59122 maize is a GM hybrid that confers resistance to lepidopteran and coleopteran pests and tolerance to the herbicidal active ingredient glufosinate-ammonium. Diets were fed *ad libitum* for at least 92 days. No significant differences were observed in nutritional performance variables, clinical and neurobehavioral signs, ophthalmology, clinical pathology (hematology, clinical chemistry, coagulation, and urinalysis), organ weights, and gross and microscopic pathology between rats in the 091 and 1507 × 59122 treatment groups. In another 13-week feeding study by the same authors (Appenzeller et al., 2009b) also conducted in Sprague–Dawley rats, the potential health effects from consumption of a diet formulated with grain from GM herbicide-tolerant maize DP-Ø9814Ø-6 (98140; trade name Optimum GAT) were evaluated. Maize event 98140 expresses the GAT4621 (glyphosate acetyltransferase) and ZM-HRA (modified version of a maize acetolactate synthase) proteins. The first one, encoded by the *gat4621* gene, is responsible for confering plant tolerance to glyphosate-containing herbicides by acetylating glyphosate and thereby rendering it non-phytotoxic whereas the ZM-HRA protein, encoded by the *zm-hra* gene, confers tolerance to the ALS-inhibiting class of herbicides (Appenzeller et al., 2009b). Compared with rats fed diets containing grain from the conventional near-isogenic control maize, no adverse effects were observed in animals fed diets containing grain from 98140 or 98140 + Gly/SU (treated with herbicides containing the active ingredients glyphosate and nicosulfuron plus rimsulfuron) maize with respect to standard nutritional performance metrics and OECD 408-compliant toxicological response variables. In both studies (Appenzeller et al., 2009a,b), the authors concluded that 1507 × 59122 maize grain and Optimum GAT were as safe and nutritious as non-GM maize grain.

In mice, Juberg et al. (2009) did not find evidence of acute toxicity following oral exposure to either the Cry34Ab1 or Cry35Ab1 proteins individually or concomitantly. Similarly, no adverse effects were observed in a repeated dose (28 day) dietary toxicity study that incorporated these proteins into diets at concentrations corresponding up to 1000-fold greater than the highest estimate of human exposure based on the concentrations of these proteins expressed in 59122 maize grains (Juberg et al., 2009). According to the authors (Juberg et al., 2009), these studies demonstrated that the Cry34Ab1 and Cry35Ab1 proteins did not represent a risk to human health and supported previous studies indicating that 59122 maize grain is as safe and wholesome as non-GM maize grain. Expression of the Cry34Ab1 and Cry35Ab1 proteins from Bt Berliner strain PS149B1 in GM maize (event DAS-59122-7) protects the crop from damage due to feeding by *Diabrotica* larvae including the western corn rootworm (*Diabrotica virgifera* virgifera). On the other hand, other researchers (McNaughton et al., 2007) did not observe statistically significant differences in mortality, growth performance variables, or carcass and organ yields between broilers consuming diets containing transgenic maize grains from event DP-Ø9814Ø-6 (Optimum GAT), near-isogenic control maize grain, or commercial reference maize grains. It must be noted that in this study adverse/toxic effects of the transgenic maize were not investigated given that the study was mainly conducted to mimic some variables that would be normally measured by commercial poultry producers.

Recently, two 90-days feeding studies (He et al., 2008, 2009) were conducted in Sprague–Dawley rats, to which grain from corn rootworm resistant transgenic DAS-59122-7 maize, and transgenic lysine-rich maize grain (Y642) were given. The results were compared with those obtained from rats given non-transgenic maize. In the first study (He et al., 2008), significant differences were observed in certain hematology and serum chemistry response variables between rats consuming diets formulated with 59122 compared to AIN93G diet (a commercial diet used as control). However, the authors concluded that these differences were related to consumption of diets containing high concentrations of maize flour (compared to AIN93G diets) regardless of source, rather than to consumption of flour from 59122 maize grain. Therefore, it was concluded that 59122 maize grain was as safe as non-transgenic maize grain (He et al., 2008) and hence in accordance with that reported by Malley et al. (2007) although using different experimental designs.

On a similar approach, following studies (He et al., 2009) showed no adverse diet-related differences in body weights, feed consumption/utilization, clinical chemistry, hematology, and absolute and relative organ weights between rats consuming diets with Y642 maize grain compared with rats consuming diets containing Nongda 108 maize grain (near-isogenic non-GM quality protein maize). Maize event Y642 has kernels enriched in lysine content primarily aiming to improve monogastric animal nutrition whereas Nongda 108 maize, used in the above-mentioned study as a control, is a high-lysine corn obtained by conventional breeding. No differences in gross or microscopic pathology were observed and according to the authors (He et al., 2009), these results demonstrate that Y642 lysine-rich maize was as safe and nutritious as conventional quality protein maize.

Other groups of investigators have also evaluated the safety of GM maize/corn grains. For instance, Healy et al. (2008) performed a 13-week rat feeding study with grain from MON 88017 corn (brand name YieldGard VT Rootworm/RR2), protected from feeding damage caused by corn rootworm and tolerant to glyphosate, the active ingredient in Roundup agricultural herbicides. MON 88017 was formulated into rat diets at 11 or 33% (w/w) levels with its near-isogenic control at a level of 33% (w/w). Additionally, six diets containing grain from different

conventional (non-biotechnology-derived), reference hybrids were formulated, each at 33% (w/w) levels of one of six reference grains. No adverse health effects were noted. Consistent with agronomic, compositional and farm animal feeding studies, the 90-day rat study did not detect unintended effects. The authors concluded that MON 88017 was as safe and nutritious as conventional corn hybrids. Other researchers (Herouet-Guicheney et al., 2009) assessed the potential safety concerns related to the transgenic 2mEPSPS (5-enol pyruvyl-shikimate-3-phosphate synthase), a protein with a lower binding affinity for glyphosate, which is highly resistant to the inhibition by glyphosate, and thus allows sufficient enzyme activity for the plants to grow in the presence of herbicides that contain glyphosate. The safety evaluation supported that the expressed protein was innocuous. The 2mEPSPS enzyme did not possess any of the properties associated with known toxins or allergens, including a lack of amino acid sequence similarity to known toxins and allergens, a rapid degradation in simulated gastric and intestinal fluids, and no adverse effects in mice after intravenous or oral administration (at 10 or 2000 mg/kg body weight, respectively). It was concluded that there was a reasonable certainty of no harm resulting from the inclusion of the 2mEPSPS protein in human food or in animal feed.

In the scientific literature, there also exist various references concerning studies performed by Russian investigators (Tutel'ian et al., 2008, 2009; Tyshko et al., 2008, 2009). These authors assessed medical and biological safety of GM maize rootworm *Diabrotica* spp.-protected event MIR604 and rootworm *Diabrotica* spp.-protected and glyphosate-tolerant maize event MON 88017. Analysis of morphological, hematological and biochemical parameters and system (sensitive) biomarkers did not reveal any toxic effect of maize event MIR604 and MON 88017 (Tutel'ian et al., 2008, 2009), while analysis of damages of DNA and structural chromosome aberrations and assessment of the allergenic potential and immunoreactive properties did not show any genotoxic, allergenic and immunotoxic effect of those GM corns (Tyshko et al., 2008, 2009). Nevertheless, and considering that these four references (Tutel'ian et al., 2008, 2009; Tyshko et al., 2008, 2009) are in Russian, only information from the abstracts was included in the present review.

2.2. Rice

The most recent studies concerning safety of GM-rice have been performed as a part of the SAFOTEST project by the group headed by Dr. Knudsen from the Danish Institute for Food and Veterinary Research. SAFOTEST is an EU project designed to develop scientific methodologies for assessing the safety of GM crops, being the 90-day animal study the core study for the safety assessment of GM foods (Poulsen et al., 2007a). Accordingly, in a 90-day feeding study on Wistar rats (Schrøder et al., 2007), the authors compared the transgenic KMD1 rice expressing Cry1Ab protein (Bt toxin) to its non-transgenic parental wild type, Xiushui 11. The KMD1 rice contained 15 mg Bt toxin/kg, and based on the average feed consumption, the daily intake was 0.54 mg Bt toxin/kg body weight. No adverse effects on animal behavior or weight gain were observed during the study. A few hematological and biochemical parameters (analyzed from blood samples collected 1 week prior to sacrifice) were significantly different. Nonetheless, all were within the normal reference intervals for rats of this breed and age, and consequently not considered treatment related. Upon sacrifice, a number of organs were weighed, and macroscopic and histopathological examinations were performed. Only minor changes were observed (Schrøder et al., 2007). Although the results showed no adverse or toxic effects of KMD1 rice when tested in the 90-day study, the authors indicated that based on the experiences from that investigation, safety assessment for unintended effects of a GM crop could not be done without additional test group(s). In another feeding study conducted by the same research group (Poulsen et al., 2007b), Wistar rats were given a purified diet containing either 60% of a rice variety expressing the snowdrop *Galanthus nivalis* lectin (GNA lectin), or parental rice for 90 days. A range of clinical, biological, immunological, microbiological and pathological parameters were examined, with a number of significant differences observed between groups fed the two diets. Although none of them was considered to be adverse, the authors remarked that the design of their study was not able to conclude on the safety of the GM food. As in an earlier study (Schrøder et al., 2007), it was suggested that additional group(s), where the expressed gene products have been spiked to the diet, should be included in order to be able to distinguish whether the observed effects were due to the GNA lectin per se or to secondary changes in the GM-rice. Besides, as part of the SAFOTEST project, the immunomodulating effect of Cry1Ab protein from Bt and *Phaselous vulgaris* lectin agglutinin E-form (PHA-E lectin) from kidney bean was examined in 28- and 90-day feeding studies in Wistar rats. Animals were fed control rice, transgenic rice expressing Cry1Ab protein or PHA-E lectin, or transgenic rice spiked with the purified recombinant protein (Kroghsbo et al., 2008). Total immunoglobulin levels, mitogen-induced cell proliferation, T-dependent antibody response to sheep red blood cells, and the antigen-specific antibody response in serum were examined at the end of the studies. A dose-dependent increase in mesenteric lymph node weight and total immunoglobulin A was seen when feeding PHA-E transgenic rice alone or spiked with 0.1% purified PHA-E lectin for 90 days indicating a local effect of PHA-E in the intestine. No adverse effects of Cry1Ab protein were found, while an anti-PHA-E and anti-Cry1Ab antibody response was induced both after inhalation (control groups) and after inhalation/ingestion (groups fed recombinant protein alone or together with transgenic rice). In conclusion, only PHA-E lectin was found to have an immunomodulating effect when feeding rats for 90 days with approximately 70 mg PHA-E/kg body weight per day.

Recently, Domon et al. (2009) reported the results of the first oral long-term safety assessment of transgenic plant products containing 7Crp (seven major human T-cell epitopes derived from Japanese cedar pollen allergens, which might be exploited to control pollen allergy in humans) using nonhuman primates (*Cynomolgus macaques*) over 26 weeks. Specifically, monkeys were orally administered a high or low dose of transgenic rice containing 7Crp or the non-transgenic control by gavage every day. No adverse effects on general behavior or body weight of animals were observed during the study, while analysis of blood from primates administered for 26 weeks showed that, with few exceptions, there were no significant differences in hematological or biochemical values between them. Moreover, neither pathological symptoms nor histopathological abnormalities were seen. It was concluded that oral administration of transgenic rice containing T-cell epitopes from Japanese cedar pollen allergens had no adverse effects and were safe when eaten every day (Domon et al., 2009).

2.3. Soybeans

With respect to recent studies on safety assessment of GM soybeans, the scientific literature shows rather contradictory results. Two research groups have been especially active in relation to those investigations. One of them, headed by Dr. Delaney from Pioneer Hi-Bred International, Inc. (Johnston, IA, USA), has reported data showing that various GM soybeans were safe. In contrast, the group headed by Dr. Malatesta from the University of Verona (Verona, Italy) has shown notable concerns. A summary of recent studies is next presented.

In Sprague–Dawley rats, Appenzeller et al. (2008) conducted a subchronic feeding study with the herbicide-tolerant soybean DP-356Ø43-5 (356043). Diets were fed to young adult animals for at least 93 days. Compared with rats fed the isoline control or conventional reference diets, no biologically-relevant, adverse effects were observed in rats fed diets containing 356043 soybean with respect to body weight/gain, food consumption/efficiency, clinical signs, mortality, ophthalmology, neurobehavioral assessments (sensory response, grip strength and motor activity), clinical pathology (hematology, coagulation, serum chemistry and urinalysis), organ weights, and gross and

Table 1
A summary of experimental studies concerning dietary administration of genetically modified plants to various animal species.

Plant/crop	Animal species	Length of study	Main adverse effects	Reference
Corn/maize				
MON 863	Rats	90 days	Slight but dose-related weight variations in both males (3.3% reduction) and females (3.7% increase). Signs of hepatorenal toxicity, increased triglycerides in females (24–40%) and urine phosphorus and sodium excretions diminished in males (31–35%)	Séralini et al. (2007)
MON 863[a]	Rats	90 days	No evidence of adverse effects	Doull et al. (2007)
NK 603, MON 810 and MON 863	Rats	14 weeks	Sex- and dose-dependent side effects linked with consumption of 3 GMOs and mostly associated with hepatorenal toxicity. Other adverse effects were also detected in heart, spleen, adrenal glands and hemopoietic system	de Vendômois et al. (2009)
Maize 1507	Sprague–Dawley rats	90 days	No significant differences were observed in nutritional performance variables, clinical and neurobehavioral signs, ophthalmology and clinical pathology, organ weights and gross and microscopic pathology between treatment groups	MacKenzie et al. (2007)
Maize 59122	Rats	90 days	No adverse diet-related differences in body weight, food consumption, clinical signs of toxicity, mortality, ophthalmology, neurobehavioral assessments, clinical pathology and pathology	Malley et al. (2007)
Maize 1507 × 59122	Sprague–Dawley rats	92 days	No significant differences were observed in nutritional performance variables, clinical and neurobehavioral signs, ophtalmology and clinical pathology, organ weights and gross and microscopic pathology between treatment groups	Appenzeller et al. (2009a)
Maize DP-Ø9814Ø-6	Sprague–Dawley rats	13 weeks	No adverse effects were observed in nutritional performance variables and OECD 408-compliant toxicological response variables	Appenzeller et al. (2009b)
Maize 59122[b]	Mice	28 days	No signs of acute toxicity or adverse effects due to diets containing high concentrations of Cry34Ab1 or Cry35Ab1 proteins, individually or concomitantly, were found at concentrations nearly 1000-fold greater than those found in 59122 maize grains	Juberg et al. (2009)
Maize DP-Ø9814Ø-6	Broilers	42 days	No significant differences in mortality, growth performance variables or carcass and organ yields. Adverse-toxic effects of the transgenic maize were not assessed.	McNaughton et al. (2007)
DAS-59122-7	Sprague–Dawley rats	90 days	Significant differences in certain hematology and serum chemistry response variables, but attributed to diets containing high maize flour (compared to control diets). It was concluded that 59122 maize grains were as safe as non-transgenic maize diets	He et al. (2008)
Y642 (lysine-rich)	Sprague–Dawley rats	90 days	No adverse diet-related adverse effects in body weight, feed consumption, clinical chemistry, hematology, and absolute and relative organ weights	He et al. (2009)
MON 88017	Rats	13 weeks	No adverse health effects were noticed.	Healy et al. (2008)
Maize (2mEPSPS)	Mice	–	The safety evaluation concluded that the protein was innocuous and hence could be included in human food or animal feed.	Herouet-Guicheney et al. (2009)
MIR 604, MON 88107	–	–	Analysis of morphological, hematological and biochemical parameters and system sensitive biomarker did not reveal any toxic effect.	Tutel'ian et al. (2008, 2009)
MIR 604, MON 88107	–	–	Analysis of DNA damage and structural chromosome aberrations, assessment of allergenic potential and immunoreactive properties did not show any genotoxic, allergenic and immunoreactive effects.	Tyshko et al. (2008, 2009)
Rice				
KMD1	Wistar rats	90 days	No adverse effects on animal behavior or weight gain. Few hematological and biochemical parameters were significantly different between treatment diets. However, all were within the normal reference intervals for rats of this breed and age. Minor changes were observed in organs weight and macroscopic and histopathological examinations.	Schrøder et al. (2007)
Rice expressing GNA lectin	Wistar rats	90 days	No adverse effects were observed. However, a range of clinical, biological, i mmunological, microbiological and pathological parameters were significantly different between diet groups. The authors remarked that the design of their study was not able to conclude on the safety of the product.	Poulsen et al. (2007a,b)
Rice expressing Cry1Ab protein or PHA-E lectin	Wistar rats	28- and 90-days	A dose-dependent increase in mesenteric lymph node weight and total immunoglobulin A was seen when feeding PHA-E transgenic rice alone or spiked with 0.1% purified PHA-E lectin for 90 days. No adverse effects of Cry1Ab protein were found.	Kroghsbo et al. (2008)
Rice containing 7Crp	*Cynomolgus macaques*	26 weeks	No adverse effects on general behavior or body weight, hematological and biochemical variables. No pathological symptoms or histopathological abnormalities.	Domon et al. (2009)
Soybeans				
DP-356Ø43-5	Sprague–Dawley rats	>93 days	No adverse effects on body weight/gain, food consumption, clinical signs, mortality, ophthalmology, neurobehavioral assessment, clinical pathology, organ weights and gross and microscopic pathology	Appenzeller et al. (2008)
DP-356Ø43-5	Broilers	42 days	No adverse effects were found. It was concluded that GM 356Ø43 was nutritionally equivalent to non-GM soybean with comparable genetic background	McNaughton et al. (2008)
DP-3Ø5423-1	Sprague–Dawley rats	–	No adverse effects on body weight/gain, food consumption, and mortality, clinical signs of toxicity or ophthalmological observations, neurobehavioral assessments, organ weights or clinical and anatomic pathology	Delaney et al. (2008)
HRA	Mice	28 days	No adverse effects	Mathesius et al. (2009)
Soybean expressing CP4 EPSPS gene	Mice	–	Several proteins belonging to hepatocyte metabolism, stress response, calcium signaling and mitochondria were differentially expressed in	Malatesta et al. (2008a)

(continued on next page)

Table 1 (*continued*)

Plant/crop	Animal species	Length of study	Main adverse effects	Reference
			GM-fed mice indicating a more marked expression of senescence markers in comparison to controls. GM-fed mice showed mitochondrial and nuclear modifications indicative of reduced metabolic rate	
GM	Mice	–	No morphological differences in embryos of GM and non-Gm soybean-exposed groups. Microscopic and ultramicroscopic cellular changes attributed to GM soybean intake	Cisterna et al. (2008)
SUPRO 500E	Wistar rats	30 days	No adverse effects in nutritional performance. Altered pancreas function evidenced by the early acute PAP mRNA increased levels and pancreas cellular changes	Malatesta et al. (2002a,b)
Glyphosphate tolerant	F344 rats	52 weeks	No adverse effect in gross necropsy findings, hematological and serum biochemical parameters, organ weights and pathological findings	Sakamoto et al. (2007)
Glyphosphate tolerant	F344 rats	104 weeks	No adverse effect in gross necropsy findings, hematological and serum biochemical parameters, organ weights and pathological findings	Sakamoto et al. (2008)

[a] Expert panel convened to assess the original study results analyzed by Montsanto Company and the reanalysis conducted by Séralini et al. (2007).
[b] Oral exposure to either the Cry34Ab1 or Cry35Ab1 proteins found in 59122 maize.

microscopic pathology. In a 42-day feeding trial study conducted in broiler chickens (McNaughton et al., 2008), it was also concluded that 356043 soybean was nutritionally equivalent to non-transgenic control soybean with a comparable genetic background. Delaney et al. (2008) carried out in Sprague–Dawley rats a subchronic feeding study of high oleic acid soybeans (Event DP-3Ø5423-1). DP-3Ø5423-1 (305423) is a GM soybean produced by biolistic insertion of a gm-fad2-1 gene fragment and the gm-hra gene into the germline of soybean seeds. Compared with rats fed the non-GM control diet, no biologically-relevant differences were observed in animals fed the 305423 diet with respect to body weight/gain, food consumption/efficiency, mortality, clinical signs of toxicity, or ophthalmologic observations. In addition, no diet-related effects were noted on neurobehavioral assessment, organ weights, or clinical or anatomic pathology. Based on the results of these studies, the authors concluded that 356043 and 305423 soybeans were as safe and nutritious as conventional non-GM soybeans (Appenzeller et al., 2008; Delaney et al., 2008). Also related to GM soybeans, Mathesius et al. (2009) assessed the safety of a modified acetolactate synthase protein (GM-HRA) used as a selectable marker in GM soybeans. The authors (Mathesius et al., 2009) did not find adverse effects in mice following acute oral exposure to GM-HRA at a dose of at least 436 mg/kg of body weight, or in a 28-day repeated dose dietary toxicity study at doses up to 1247 mg/kg of body weight/day. It was concluded that GM-HRA protein is safe when used in agricultural biotechnology.

In contrast to the above results, in a long-term study on female mice fed a GM modified soybean (insertion of the bacterial CP4 EPSPS gene to confer a high level of tolerance to glyphosate), focused on assessing the effects of this diet on liver of old animals (until 24 months of age) and to elucidate possible interference with aging, Malatesta et al. (2008a) found that GM soybean intake could influence the liver morpho-functional features during the physiological process of aging. Several proteins belonging to hepatocyte metabolism, stress response, calcium signaling and mitochondria were differentially expressed in GM-fed mice, indicating a more marked expression of senescence markers in comparison to controls. Moreover, hepatocytes of GM-fed mice showed mitochondrial and nuclear modifications indicative of reduced metabolic rate. In previous studies on hepatocytes from young and adult (2–8 months of age) female mice fed GM soybeans, nuclear modifications involving structural constituents of the transcription and splicing properties pathways were seen (Malatesta et al., 2002a). Although the cause(s) of the observed alterations could not be conclusively established, it was noted that these modifications disappeared when GM soybean was replaced by a non-GM one in the diet (Malatesta et al., 2005). Since the GM soybean used was tolerant to glyphosate and was treated with the glyphosate-containing herbicide Roundup, the effects observed might be due to herbicide residues. Accordingly, and aiming to verify this hypothesis, Malatesta et al. (2008b) treated rat hepatoma tissue culture (HTC) cells with 1–10 mM Roundup and analyzed cellular features by flow cytometry, fluorescence, and electron microscopy. Under these experimental conditions, the death rate and the general morphology of HTC cells were not affected, as well as most of the cytoplasmic organelles. However, in HTC-treated cells, lysosome density increased and mitochondrial membranes were modified indicating a decline in the respiratory activity. In addition to the above, nuclei underwent morpho-functional modifications suggesting a decreased transcriptional/splicing activity. The authors did not exclude that factors other than the presence of the herbicide residues could be responsible for the cellular modifications described in GM-fed mice. However, they indicated that the concordance of the effects induced by low concentrations of Roundup on HTC cells suggested that the presence of Roundup residues could be one of the factors interfering with multiple metabolic pathways.

Cisterna et al. (2008) investigated the ultrastructural and immunocytochemical features of pre-implantation embryos from mice fed either GM or non-GM soybean in order to verify whether the parental diet could affect the morpho-functional development of the embryonic ribonucleoprotein structural constituents involved in pre-mRNA pathways. Morphological observations revealed that the general aspect of embryo nuclear components were similar in the GM and non-GM soybean-exposed groups. However, immunocyto-chemical and in situ hybridization results suggested a temporary decrease of pre-mRNA transcription and splicing in 2-cell embryos and a resumption in 4–8-cell embryos from mice fed GM soybean. In addition, pre-mRNA maturation seemed to be less efficient in both 2-cell and 4–8-cell embryos from GM-fed mice than in non-GM-fed animals. In a previous ultrastructural analysis of testes from mice fed GM soybean conducted by the same research group (Vecchio et al., 2004), it was found that the immunolabelling for Sm antigen, hnRNPs, SC35 and RNA Polymerase II was decreased in 2 and 5 month-old GM-fed mice, and was restored to normal at 8 months. In GM-fed mice of all ages considered, the number of perichromatin granules was higher and the nuclear pore density lower. Moreover, enlargements in the smooth endoplasmic reticulum in GM-fed mice Sertoli cells were also observed. Consequently, the studies by the Malatesta's group (Malatesta et al., 2005, 2008b; Cisterna et al., 2008) at the microscopic and ultramicroscopic levels showed cellular changes attributable to GM soybean intake.

Magaña-Gómez et al. (2008) conducted a study in Wistar rats, in which the hypothesis was that the intake of GM (SUPRO 500E) soybean could induce pancreatic stress or injury by analyzing the expression of pancreatitis-associated protein (PAP) and trypsinogens

by qRT-PCR in rats fed GM soy protein for 30 days. The hypothesis was based on the results of previous investigations showing that mice chronically fed since gestation with GM had problems in synthesis and processing of zymogens by pancreatic acinar cells and reduced nucleoplasmic and nucleolar and perichromatin granule accumulation on pancreatic acinar cell nuclei (Malatesta et al., 2002b, 2003). Magaña-Gómez et al. (2008) did not find differences in nutritional performance among rats fed non-GM and GM diets. The GM diet induced significant zymogen-granule depletion after 15 days feeding, returning to normal levels after 30 days. Acinar disorganization started as early as 5 days after initiation of the GM diet and it recovered after 30 days. Levels of PAP mRNA significantly increased in the GM diet between day 1 and day 3 and decreased to the basal level by day 15. In turn, trypsinogen mRNA peaked at two different times: at day 1 and at day 15, decreasing to basal levels after 30 days, while plasma amylase levels remained unchanged at all times. The authors indicated that GM soy protein intake affected pancreas function, evidenced by the early acute PAP mRNA increased levels and pancreas cellular changes followed by recuperation of acinar cells after 30 days. In Japan, Sakamoto et al. (2007, 2008) conducted 52-week and 104-week feeding studies of genetically modified soybeans in F344 rats. Although in both studies several differences in animal growth, food intake, serum biochemical parameters and histological findings were observed between rats fed the GM (glyphosate-tolerant) soybeans and those fed a commercial diet, body weight and food intake were similar for the rats fed the GM and non-GM soybeans. Gross necropsy findings, hematological and serum biochemical parameters, organ weights, and pathological findings showed no meaningful differences between rats fed the GM and non-GM soybeans. These results indicate that long-term intake (54 and 104 weeks) of GM soybeans at the level of 30% in the diet had no apparent adverse effect in rats.

3. Final remarks

In the same line of our previous papers (Domingo, 2000, 2007; Domingo-Roig and Gómez-Arnáiz, 2000), the main purpose of this review-article was to critically revise the published scientific literature on potential toxic effects/health risks of GM plants. It was noticed that the total number of general references on GMOs in general, and GM foods/plants in particular, found in the databases PubMed and Scopus has considerably increased between our 2006 search (Domingo, 2007) and the current one. In spite of this, the number of studies specifically focused on safety assessment of GM plants is still limited. However, it is important to remark that for the first time, a certain equilibrium in the number of research groups suggesting, on the basis of their studies, that a number of varieties of GM products (mainly maize and soybeans) are as safe and nutritious as the respective conventional non-GM plant, and those raising still serious concerns, was observed. Moreover, it is worth mentioning that most of the studies demonstrating that GM foods are as nutritional and safe as those obtained by conventional breeding, have been performed by biotechnology companies or associates, which are also responsible of commercializing these GM plants. Anyhow, this represents a notable advance in comparison with the lack of studies published in recent years in scientific journals by those companies (Domingo, 2007). The scientific community may finally be able to critically evaluate and discuss all that information, which was not possible until now. Scientists know quite well how different may be the information published in reputed international journals, which has been submitted to peer-review processes, from those general comments/reports not submitted to this selective procedure.

A relatively remarkable finding of the present review is that the published scientific literature between October 2006 (Domingo, 2007) and August 2010 (current review) on edible GM plants, concerns only to three products: corn/maize, soybeans, and rice, rice being comparatively the less abundant. We have not been able to find citations involving investigations on GM potatoes (except a review by Arvanitoyannis et al., 2008), peas, tomatoes, pepper, etc., after October 2006. A summary of experimental studies (October 2006–August 2010) concerning dietary administration of those products to various animal species is shown in Table 1. With respect to corn/maize, various studies have concluded that the transgenic varieties 1507 (MacKenzie et al., 2007), 59122 (Malley et al., 2007; Juberg et al., 2009; He et al., 2008), 1507 × 59122 (Appenzeller et al., 2009a), 98140 (Appenzeller et al., 2009b; McNaughton et al., 2007), Y642 (He et al., 2009), and MON 88017 (Healy et al., 2008) were as safe as conventional quality protein maize. In contrast, Séralini's group raised concern regarding some commercialized GM maize (NK 603, MON 810 and MON 863) (Séralini et al., 2007, 2009; de Vendômois et al., 2009). Similarly, scientific controversy is also present in relation to the safety of GM soybeans. While it has been reported that 356043 (Sakamoto et al., 2007) and 305423 (Delaney et al., 2008) soybeans were as safe as conventional non-GM soybeans, some authors are still concerned by the safety of GM soybeans and recommend to investigate the long-term consequences of GM diets and the potential synergistic effects with other products and/or conditions (Malatesta et al., 2008a,b; Cisterna et al., 2008; Magaña-Gómez et al., 2008).

In the period here revised, October 2006–August 2010, a few reviews on health risks of GM foods/plants have been also published (Dona and Arvanitoyannis, 2009; Magaña-Gómez and de la Barca, 2009; Key et al., 2008). In general terms, all these authors agree in remarking that more scientific efforts are clearly necessary in order to build confidence in the evaluation and acceptance of GM foods/plant by both the scientific community and the general public. Especially critical is the recent review by Dona and Arvanitoyannis (2009), who remarked that results of most studies with GM foods would indicate that they may cause some common toxic effects such as hepatic, pancreatic, renal, or reproductive effects, and might alter the hematological, biochemical, and immunologic parameters. These authors also concluded that the use of recombinant GH or its expression in animals should be re-examined since it has been shown that it increases IGF-1 which, in turn, may promote cancer. A harsh response to that review was recently published in the same journal (Rickard, 2010). This is indeed only an example on the controversial debate on GMOs, which remains completely open at all levels.

Finally, we would like to indicate that the review on allergenicity of GM plants has not been included herein. European legislation stipulates that GMOs have to be monitored to identify potential adverse environmental effects (Reuter et al., 2010). The European Food Safety Authority (EFSA) has recently published a Scientific Opinion regarding assessment of allergenicity of GM plants and microorganisms and derived food and feed (EFSA, 2010). Detailed information on this important issue is available at http://www.efsa.europa.eu/en/scdocs/scdoc/1700.htm.

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Toxicity Studies of Genetically Modified Plants: A Review of the Published Literature

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According to the information reported by the WHO, the genetically modified (GM) products that are currently on the international market have all passed risk assessments conducted by national authorities. These assessments have not indicated any risk to human health. In spite of this clear statement, it is quite amazing to note that the review articles published in international scientific journals during the current decade did not find, or the number was particularly small, references concerning human and animal toxicological/health risks studies on GM foods. In this paper, the scientific information concerning the potential toxicity of GM/transgenic plants using the Medline database is reviewed. Studies about the safety of the potential use of potatoes, corn, soybeans, rice, cucumber, tomatoes, sweet pepper, peas, and canola plants for food and feed were included. The number of references was surprisingly limited. Moreover, most published studies were not performed by the biotechnology companies that produce these products. This review can be concluded raising the following question: where is the scientific evidence showing that GM plants/food are toxicologically safe?

Keywords genetically modified (GM) plants, toxicity, safety, health risks, DNA

INTRODUCTION

The World Health Organization (WHO) defines genetically modified organisms (GMOs) as those organisms in which the genetic material has been altered in a way that does not occur naturally (WHO, 2002). The technology used allows selected individual genes to be transferred from an organism into another, and also between non-related species. Such methods are used to create genetically modified (GM) plants, which are then used to grow GM food crops. The GM crops currently on the market are mainly aimed at an increased level of crop protection through the introduction of resistance against plant diseases caused by insects or viruses, or through increased tolerance towards herbicides.

Taking into account that different GMOs include different genes inserted in different ways, the WHO indicates that individual foods and their safety should be assessed in a case-by-case basis, and that it is not possible to make general statements on the safety of all GM foods. In general terms, the safety assessment of GM foods should investigate:

a) toxicity,
b) allergenicity,
c) specific components thought to have nutritional or toxic properties,
d) stability of the inserted gene,
e) nutritional effects associated with genetic modification, and
f) any unintended effects which could result from the gene insertion (WHO, 2002).

Although the WHO declares that the GM products that are currently on the international market have all passed risk assessment conducted by national authorities, in a review on the scientific literature performed in 2000, we were not able to find sufficient published information concerning that assessment (Domingo and Gómez, 2000). In particular, the lack of published toxicological studies on adverse health effects was evident. Although a considerable number of commentaries, general news, and letters to the Editor were published in reputable international journals, papers about experimental investigations on the safety of GM foods were surprisingly very scant. We concluded that if data on toxicological assessment of GM foods were obtained, these were not reported in scientific journals and subjected to the scientific judgment (Domingo, 2000; Domingo and Gómez, 2000).

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An important problem seems to be related to the safety assessment of new GM foods, which is initially based on the use of the concept of "substantial equivalence." This concept is based on the following principle: "if a new food is found to be substantially equivalent in composition and nutritional characteristics to an existing food, it can be regarded as being as safe as the conventional food" (SOT, 2003). Although application of the concept is not a safety assessment per se, it enables the identification of potential differences between the existing food and the new product, which should then be investigated further with respect to their toxicological impact. It is a starting point rather than an end point (Kuiper et al., 2002).

Which is the current situation concerning health risks of GM foods six years after our previous revision was performed (Domingo and Gómez, 2000)? The scientific literature on the potential adverse health/toxic effects of GM/transgenic foods has been again reviewed using the Medline database (available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed). The search covered the period January 1980-October 2006. The following "key terms" (number of references in parenthesis) were used: genetically modified foods (686), GM foods (3498), transgenic foods (4127), toxicity of transgenic foods (136), health risks of transgenic foods (23), adverse effects of genetically modified foods (170), toxicity of genetically modified foods (38), health risks of GM foods (38), health risks of genetically modified foods (72), toxicity of GM foods (120), adverse effects of GM foods (276), and adverse effects of transgenic foods (199). It can be seen that citations corresponding to general "key terms" such as: genetically modified foods, GM foods, and transgenic foods are quantitatively very important. However, references concerning specific risk assessment are much more limited. Moreover, most references corresponding to the key terms "adverse effects," "toxicity" and "health risks," did not directly correspond to the main topic of the search. A review of the published studies directly related with health risks (including toxicity) of GM plants consumed as food and/or feed is here presented. Information and details are given according to the specific plant. A summary of results concerning the most relevant studies are summarized in Table 1. With only a few exceptions, studies concerning allergenicity of GM plants were not included here. However, a system of food allergy vigilance encompassing the full range of foods consumed is clearly essential (Moneret-Vautrin et al., 2004). Those GM crops that are specifically related to food sensitivity (e.g., wheat, peanuts) are of special concern.

GM PLANTS

Potatoes

In the mid 1970s, the WHO and other international institutions initiated studies on the development of existing and new biological control agents for pest controls. The most popular of these agents are strains of *Bacillus thuringiensis*. Among these, *Bacillus thuringiensis* var. *kurstaki* was proven to produce an effective toxin against lepidopteran insects. In recent years, transgenic potatoes were produced in which the CryI gene of *Bacillus thuringiensis* var. *kurstaki*. The gene was transmitted into the plant cells via a shuttle plasmid vector after cloning in *E. Coli.* Fares and El-Sayed (1998) investigated the effect of feeding transgenic potatoes, which carry the CryI gene of *Bacillus thuringiensis* var. *kurstaki* strain HD1, on the light and electron microscopic structure of the mice ileum, in comparison with feeding potatoes treated with the "delta-endotoxin" isolated from the same bacterial strain. The microscopic architecture of the enterocytes of the ileum of both groups of mice revealed certain common features such as the appearance of mitochondria with signs of degeneration and disrupted short microvilli at the luminal surface. However, in the group of mice fed on the "delta-endotoxin," several villi appeared with an abnormally large number of enterocytes. Fifty percent of these cells were hypertrophied and multinucleated. Basal lamina along the base of the enterocytes was damaged at several foci. Several disrupted microvilli appeared in association with variable-shaped cytoplasm fragments. Some of these fragments contained endoplasmic reticulum, as well as ring-shaped annulate lamellae. In addition, the Paneth cells were highly activated and contained a large number of secretory granules. These changes might suggest that delta-endotoxin-treated potatoes resulted in the development of hyperplastic cells in the mice ileum. The authors concluded that the appearance of several multinucleated and hypertrophied enterocytes, as well as several associated cytoplasmic fragments with highly recognized annulate lamellae suggested the possible participation of feeding on the delta-endotoxin-treated potatoes in the hyperplastic development in the mice ileum. They recommended that in order to avoid any potential risks to the consumers, new types of heredity and new genetic structures must be evaluated before releasing for marketing new transgenic foods.

Because of the wide controversy and international repercussions of the results, especially remarkable was the publication of the study by Ewen and Pusztai (1999), who investigated the effects of diets containing GM potatoes expressing *Galanthus nivalis* lectin on rat small intestine. It was found that these diets had variable effects on different parts of the rat gastrointestinal tract. Some effects such as the proliferation of the gastric mucosa, were mainly attributed to the expressions of the *Galanthus nivalis* agglutinin (GNA) transgene. However, the authors suggested that other parts of the construct or the genetic transformation (or both) could also have contributed to the overall biological effects of the GNA-genetically modified potatoes, particularly on the small intestine and caecum. It was concluded that there would exist the possibility that a plant vector in common use in some GM plants could affect the mucosa of the gastrointestinal tract and exert powerful biological effects. It might also apply to GM plants containing similar constructs, particularly those containing lectins, such as soybeans or any plants expressing lectin genes or transgenes. The main concern in relation to this study was the short experimental period, 10 days. Would this

Table 1 A summary of experimental studies concerning dietary administration of a number of genetically modified plants to various animal species

Plant/crop	Animal species	Length of the study	Main adverse effects	Reference
Potatoe				
GM (delta-endotoxin treated)	mice	2 weeks	Mild changes in the structural configuration of the ileum. Potential hyperplastic development of the ileum	Feres and El-Sayed (1998)
GM	Rats	10 days	Proliferation of the gastric mucosa. Effects on the small intestine and caecum	Ewen and Pusztai (1999)
GM	Rats	4 weeks	Absence of pathologic symptoms and histopathological abnormalities in liver and kidney	Hashimoto et al. (1999a)
GM	Rats	5 weeks	Increase in the number of bacteria phagocytized by monocytes, percentage of neutrophils producing ROS, and oxygen-dependent bactericidal activity of neutrophils	Winnicka et al. (2001)
GM	Rats	10 weeks prior to mating	No adverse effects on the multigeneration reproductive-developmental ability	Rhee et al. (2005)
Maize/corn				
Transgenic Event 176 Bt	chickens	38 days	No deleterious effects were noted	Brake and Vlachos (1998)
GM	pigs	Growing phase	Toxicity was not assessed	Spencer et al. (2000a,b)
GM (Bt)	pigs	91 days (growing period)	Side effects were not observed. However, the studies did not indicate the performance of toxicological tests	Reuter et al. (2002a,b)
GM (CBH351)	rats and mice	13 weeks	No immunotoxicity was detected. No other specific toxicity tests were included	Teshima et al. (2002)
Roundup Ready®	rats	13 weeks	No adverse effects were reported on overall health, body weight, food consumption, clinical pathology parameters, organ weights, and gross and microscopic appearance of tissues	Hammond et al. (2004)
Soybeans				
Glyphosate-tolerant	rats, broiler chickens, catfish and dairy cows	4 weeks (rats and cows), 6 weeks (broilers) and 10 weeks (catfish)	No significant effects in the concentrations of nutrients and antinutrients	Hammond et al. (1996)
GM 40-3-2	rats	5 months	The hepatocyte membrane function and enzymatic activity were modified within physiological standards	Tutel'ian et al. (1999)
Glyphosate-tolerant	rats and mice	15 weeks	No adverse effects on growth and the histopathology of immune-related organs. No immunotoxic activity	Teshima et al. (2000)
Glyphosate-tolerant	pigs	growing period	The studies did not indicate the performance of toxicological tests	Cromwell et al. (2002)
Glyphosate-tolerant	rats	13 weeks	No adverse effects of GM soybean meal were seen even at levels as high as 90% of the diet	Zhu et al. (2004)
Glyphosate-tolerant	mice	gestation and lactation periods	No negative effects on fetal, postnatal, pubertal or adult testicular development	Brake and Evenson (2004)
Rice				
Transgenic (soybean glycinin gene)	rats	4 weeks	No adverse effects on the blood count, blood composition or internal organ weights. No pathological symptoms. No histopathological abnormalities in liver and kidney	Momma et al. (2000)
Transgenic (anti-herbicide gene(BAR))	mice and rats	30 days	No adverse effects on body or histopathogical alterations were noted	Wang et al. (2000)
Transgenic (cowpea trypsin inhibitor)	rats	period from lactation to sexual maturation	No maternal toxicity, embryotoxicity and teratogenicity were noted	Zhuo et al. (2004a)
Transgenic (cowpea trypsin inhibitor)	rats	90 days	Some alterations on hematological parameters	Zhuo et al. (2004b)

(Continued on next page)

Table 1 A summary of experimental studies concerning dietary administration of a number of genetically modified plants to various animal species *(Continued)*

Plant/crop	Animal species	Length of the study	Main adverse effects	Reference
Transgenic (cowpea trypsin inhibitor)	mice	30 days	No immunotoxic effects were observed. No other toxicity tests were performed	Chen et al. (2004)
Transgenic	rats	90 days	Not enough evidences were found to conclude that transgenic rice had adverse effects on the rat	Li et al. (2004b)
Transgenic KMD1	rats	90 days	Although only minor changes were detected, additional tests group(s) are required	Schroder et al. (2007)
Cucumber				
Transgenic	rats	5 weeks	No adverse effects on the growth and health status	Kosieradzka et al. (2001)
Tomatoes				
GM (Bt)	rats	90 days	Body weights and food consumption were normal. Microscopy examination of tissues did not show adverse effects	Noteborn et al. (1995)
GM (CMV)	rats and mice	30 days	No significant differences with rats fed non-GM tomatoes	Chen et al. (2003)
Sweet pepper				
GM (CMV)	rats and mice	30 days	No significant differences with rats fed non-GM sweet peppers	Chen et al. (2003)
Peas				
Transgenic	rats	10 days	No harmful effects on growth, metabolism and health were observed	Pusztai et al. (1999)
Canola				
Transgenic (GFP)	rats	26 days	No general health risks were detected including a low allergenicity	Richards et al. (2003)

period be sufficient to detect relevant toxicological changes on rats small intestine?

Hashimoto et al. (1999a) confirmed that transgenic potatoes with native and designed soybean glycinins were safe based on their almost equivalent composition to that of non-transgenic and the ready digestibility of native and designed glycinins expressed in the transgenic potatoes. However, these authors indicated that this safety was based only on the concept of "substantial equivalence." Consequently, in a subsequent investigation, laboratory animal feeding experiments were included (Hashimoto et al., 1999b). Four groups of rats fed:

(I) only a commercial diet,
(II) the diet plus non-transgenic potatoes,
(III) the diet plus transgenic potatoes with native glycinin, and
(IV) the diet plus transgenic potatoes with designed glycinin.

Rats were fed 2,000 mg/kg-weight potatoes every day by oral administration. During the period tested, rats in each group (groups II, III, and IV) grew well without marked differences in appearance, food intake, body weight, or in cumulative body weight gain. No significant differences were found in blood count, blood composition, and in internal organ weights among the rats after feeding potatoes (groups II, III, and IV) for four weeks. Necropsy at the end of the experiment indicated neither pathologic symptoms in all rats tested nor histopathological abnormalities in liver and kidney. Except for a small increase in sodium levels in serum of group III rats, in general terms there were no significant differences between rats fed non-transgenic and transgenic potatoes. In conclusion, the transgenic potatoes with glycinins were confirmed to have nearly the same nutritional and biochemical characteristics as the non-transgenic ones. Despite this conclusion, the authors remarked:

1) that the safety assessment with laboratory animals is often influenced by many undefined factors,
2) that it is also difficult to feed a relevant dose of transgenic crops,
3) that previously to extrapolate the safety of GM plants to humans, long-term feeding animals experiments (including the capability to induce malformations, alterations on the reproductive function, mutagenicity and carcinogenicity), as well as the use of cultured human cell systems are clearly necessary (Hashimoto et al., 1999b; Momma et al., 2002).

The effect of feeding GM potatoes on selected indices of non-specific resistance was investigated in rats (Winnicka et al., 2001). Genetic modification of potatoes consisted of repressing the gene encoding ADP-ribosylation factor (ARF) of protein and intensification of the 14-3-3 protein synthesis (Wilczynski et al., 1997). Two semi-synthetic iso-protein diets containing potatoes, non-modified (control diet), or subjected to genetic modification (GM, experimental diet), were used. Initial mean body weight of rats was 150 g and animals fed during 5 weeks. Feeding GM potatoes increased the number of bacteria phagocytized by monocytes, the percentage of neutrophils producing reactive oxygen species (ROS), and the oxygen-dependent bactericidal activity of neutrophils. The authors concluded that a

determination of the precise mechanism of inducing the phagocytic activity observed was required. We would add the necessity to prolong the period of feeding, which in that study was probably too short.

El-Sanhoty et al. (2004) evaluated in rats the composition, nutritional and toxicology safety of GM potato Spunta lines compared to that of conventional potato Spunta. A feeding study was done for 30 days. Four groups of rats were used.

Group (I) was fed on control basal diet,
Group (II) was fed on control diet plus 30% freeze-dried non-GM potato Spunta,
Group (III) was fed on control diet plus 30% freeze-dried GM potato Spunta, and
Group (IV) was fed on control diet plus 30% freeze-dried GM potato Spunta GMO G3.

During the period tested, rats in each group (I, II, III, IV) grew well without marked differences in appearance. No significant differences were found in food intake, daily body weight gain, and feed efficiency. However, there was a slightly significant difference in finally body weight between the control and the experimental groups. No significant differences were found in serum biochemical values between groups, and also between relative organ (liver, spleen, heart, kidney, testes) weights. Although the results of this safety evaluation did not show significant differences among groups, our main concern regarding the potential extrapolation to humans of the results is again the short duration of the feeding study. Moreover, since detoxification systems in rodents are largely different from those in humans in activity and amount, as well as in the detoxification enzyme species, there would have been some additional difficulties in extrapolation of the results of animal experiments to humans (Momma et al., 2002). This comment would be appropriate not only for the study by El-Sanhoty et al. (2004), but also for any of the above studies in rodents.

A multigeneration reproductive and developmental toxicity study of the bar gene inserted into GM potatoes was recently performed in rats (Rhee et al., 2005). In each generation, animals were fed a solid pellet containing 5% GM potato and non-GM potato for 10 weeks prior to mating. In the multigeneration study, there were no GM-potato related changes in body weight, food consumption, reproductive performance, and organ weight. In each generation, the litter-related indexes did not show any GMO-related changes.

Maize/Corn

The first-commercial-scale plantings of insect-protected field corn hybrids, commonly referred to as "Bt" corn, occurred in 1996, following regulatory review by USA and Canadian authorities. These first field corn hybrids derived from a genetic modification designated "Event 176," which expresses a gene that enables the plants to produce an insecticidal protein, Cry1Ab, similar to that produced in the nature by certain subspecies of the common soil bacterium *Bacillus thuringiensis*. To determine whether transgenic Event 176-derived corn had an adverse effect on broiler chicken performance, Brake and Vlachos (1998) performed a 38-day feeding study in males and females. No statistically significant differences in survival and body weight were observed between animals reared on mash or pelleted diets prepared with transgenic corn and similar diets prepared using control corn. Broilers raised on diets prepared from the transgenic corn exhibited significantly better feed conversion ratios and improved yield of the Pectoralis minor breast muscle. Although it was not evident whether this enhanced performance was attributable to the transgenic corn per se, or due to possible slight differences in overall composition of the formulated diets, in that study that the transgenic corn had no deleterious effects.

A genetically modified corn hybrid homozygous for the lpa1 allele, containing low phytate (LP), and its nearly isogenic equivalent hybrid (normal) were compared in two experiments with growing-finishing swine (Spencer et al., 2000a). In the first experiment, 210 barrows (27 kg) were allotted to one of six dietary treatments with two corn hybrids (LP and normal) and three phosphorus (P) feeding regimens. Pigs fed the LP corn diet without added P had greater body weight gain, feed efficiency, breaking load (BL), and ash content of the fourth metacarpal than pigs fed the normal corn diet without added P. Performance was similar between pigs fed the LP diet without added P and pigs fed LP and normal corn with added P. In a second experiment with different diets, no significant differences in growing-finishing performance or BL among treatments were noted. However, pigs fed diets containing LP corn possessed carcasses with less back fat and a higher percentage of lean. These results confirmed that the P in LP corn was available to the pig and suggested that pigs fed diets containing this GM corn would have more desirable carcasses. In turn, these results corroborated previous findings of the same research group, which showed that low-phytate corn contained at least 5 times as much available P as normal corn (Spencer et al., 2000b), and suggested that low-phytate corn diets with no supplemental P might be adequate for growing-finishing swine. No toxicity experiments were included in these short-term investigations.

Studies with Bt maize in pig nutrition were also performed by Reuter et al. (2002a,b). In a first study, the composition of parental and transgenic (Bt) maize grain and its digestibility and nutritional value of both maize lines in pigs were investigated (Reuter et al., 2002a). It was concluded that from the point of view of a nutritional assessment, the GM maize could be regarded as substantially equivalent to the parental maize line. In a second study, a grower-finisher performance trial was designed to compare the growth performance of pigs fed diets containing either GM Bt-maize (NX6262) or its parental maize (Prelude) line. During a 91 days growing period, the pigs of both groups recorded equal performance in daily weight gain depending on equal amounts of feed intake (parental vs. transgenic). These results confirmed equal performance among growing-finishing pigs fed parental or GM maize containing diets. It was concluded

that diets containing a high proportion of either GM Bt maize or its non-modified parental counterpart could be fed to growing-finishing pigs without significant differences on feed consumption, daily weight gain, and energy efficiency. Unitended or unexpected side effects of the GM maize grain were not observed (Reuter et al., 2002b). However, it is important to note that there was no indication about the performance of toxicological tests in those studies.

Subchronic animal feeding studies to examine the effect on the immune system of genetically modified corn CBH351, which contains the Cry9C protein derived from *Bacillus thuringiensis* subspecies *tolworthi*, were conducted in female BN rats and B10A mice by Teshima et al. (2002). The studies were designed to compare the effect of a line of genetically modified corn CBH351 (GM corn) with that of isoline corn (non-GM corn). The study duration was 13 weeks. The following results were obtained:

(1) no remarkable compositional differences in fatty acids, amino acids or phytate were found between the GM and non-GM corns,
(2) no significant differences in growth, food intake, or weight of the thymus, spleen, and liver were found between animals fed the non-GM and GM lines,
(3) the histological findings in thymus, spleen, mesenteric lymph nodes, Peyer's patches, small intestines, liver, kidney, and bone marrow were similar in animals fed GM and non-GM lines, and
(4) no evidence of production Cry9C-specific IgE (specific marker of allergenicity) or IgA antibodies were detected in the serum of either group, whereas a minor increase of Cry9C-specific IgG (marker of exposure to the new protein) was found in the serum of rats fed 50% GM corn, but not in those fed 5% GM corn.

In conclusion, no immunotoxic activity was detected in the GM-corn-fed rats and mice in this subchronic dietary study. Although this was an extensive study concerning immunotoxicity of GM corn, again no specific toxicity tests were included.

One of the few published investigations performed by the biotechnology companies involved in commercially available GM foods is that reported by Hammond et al. (2004). These authors carried out a 13 week feeding study in rats with grain from Roundup Ready® (Monsanto, USA) corn which is tolerant to the herbicide glyphosate. The responses of rats fed diets containing Roundup Ready corn grain were compared to those of rats fed diets containing non-transgenic grain (controls). All diets were nutritionally balanced and conformed to Purina Mills, Inc. specifications for Certified LabDiet 5002. There were 400 rats in the study divided into 10 groups of 20 rats/sex/group. Overall health, body weight, food consumption, clinical pathology parameters (hematology, blood chemistry, and urinalysis), organ weights, and gross and microscopic appearance of tissues were comparable between groups fed diets containing Roundup Ready and control corn grain. The no-observed-effect level (NOEL) was equal to the highest dietary level (33%) of Roundup Ready corn grain fed to rats. According to the authors, this study complements extensive agronomic, compositional, and farm animal feeding studies with Roundup Ready corn grain, confirming it is as safe and nutritious as existing commercial corn hybrids. Although the study is extensive and seems to be well-elaborated, a potential limitation is the relatively short time of GM corn administration, 13 weeks.

On the other hand, the mineral and phytic acid contents of a low-phytic acid "flint" maize (LPM) and its parent, wild-type strain (WTH), were evaluated. Iron absorption from tortillas prepared with each type of maize and from a reference dose of ferrous ascorbate were also measured (Mendoza et al., 1998). It was found that consumption of genetically modified, low-phytic acid strains of maize, might improve iron absorption in human populations that consume maize-based diets, including those that are dependent primarily on plant-derived diets.

Soybeans

In 1996, Padgette and co-workers reported the results of extensive compositional analyses that demonstrated that glyphosate-tolerant soybeans (GTS) seeds were substantially equivalent to the commercial parental soybean variety. In another study of the same research group, the safety of the protein expression product of the cloned gene, 5-enolpyruvylshikimate-3-phosphate synthase from Agrobacterium sp. Strain CP4 (CP4 EPSPS), which is highly resistant to inhibition by glyphosate, was determined in mice (Harrison et al., 1996). There were no treatment-related adverse effects in animals given CP4 EPSPS protein by gavage at dosages up to 572 mg/kg of body weight. This dose represents a significant (greater than 1,000 fold) safety margin relative to the highest potential human consumption of CP4 EPSPS protein and assumes that the protein is expressed in multiple crops. However, these results showed that the CP4 EPSPS protein was not toxic to mammals only following acute exposure.

Although the compositional studies confirmed the equivalence of GTS to commercial soybean varieties, animal feeding trials were undertaken to provide further support for this new soybean variety. Animal feeding studies were conducted with rats, broiler chickens, catfish, and dairy cows as part of a safety assessment program. Two GTS lines and a parental variety were utilized in all animal feeding studies. The growth and gain-to-feed performance of animals fed GTS meal sources was comparable to those of animals fed parental-line soybeans. No meaningful differences between the parental and GTS lines were noted in the concentrations of important nutrients and antinutrients (Hammond et al., 1996). However, although the authors concluded that the introduced protein was safe, the period of administration was probably too short to draw convincing conclusions, as it ranged from 4 weeks for rats and dairy cows to 10 weeks for catfish. Moreover, typical toxicological parameters

were not evaluated. On the other hand, Shirai et al. (1998) reported that GTS formed approximately 1.1% of the commercial soybeans, when commercially available soybeans were cultivated and the number of soybeans resistant to glyphosate was found. This level was somewhat lower than an estimated value announced officially on the basis of the cultivation area of the GTS.

Tutel'ian et al. (1999) fed rats with albuminous concentrate from the genetically modified soybean 40-3-2 (Monsanto Co., USA), 1.25 g/rat/day for 5 months. Blood, urine, and liver were investigated to measure total protein and glucose levels, aminotransferase and alkaline phosphatase activities in blood, pH, relative density and creatinine level in the urine, and hepatic enzyme activity of the I and II phases of xenobiotic metabolism, as well as the whole and non-sedimentated lysosomal enzyme activities. It was found that the addition of the GM soybean to the diet of rats modified the hepatocyte membrane function and enzymatic activity within physiological standards, while it was not harmful to the adaptation systems.

The effect of GM and non-GM soybeans on the immune system of BN rats and B10A mice was investigated by Teshima et al. (2000). The studies were designed to compare the feeding value of a line of GM GTS to that of closely-related and one-parent same cultivar (non-GM soybeans). The study duration was 15 weeks. Growth, feeding value, and the histopathology of immune-related organs showed no significant differences between animals fed GM and non-GM lines. The production of soybean-specific IgE was not detected in the serum of any group, and the increase in soybean-specific IgG was identical in the GM and non-GM groups. No immunotoxic activity was found in GM-soybean-fed rats or mice. Some limitations of that study are the reduced number of animals per group, five, as well as the relatively short experimental period, 15 weeks.

Phipps et al. (2002) fed a GM crop to lactating dairy cows to determine if GM DNA could be detected in the milk produced by those cows. In study weeks 4–12 the total mixed ration of forage (non-GM grass and maize) was replaced by soybean meal at 26.1% of the total diet in weeks 4–5, and 13.9% of the total diet in weeks 6–12. Weekly milk samples were taken from all cows. The results showed that transgenic DNA could not be detected in milk from cows receiving up to 26.1% of their diet as herbicide ghlyphosate-tolerant soybean meal. The detection limits for the test was established at 7.5 μg/l of milk. It was suggested that an extensive degradation of DNA occurred, which would be attributed to the aggressive and extensive digestion process in the dairy cow, which was reviewed by Beever and Kemp (2000). The authors remarked that even if fragments of transgenic DNA had been detected in their study, it must be taken into account that the WHO (1993) concluded that there was no inherent risk in consuming DNA, including that from GM crops.

Recently, the health safety of transgenic soybeans (glyphosate-tolerant or Roundup Ready) was studied using the mammalian testis (mouse model) as a sensitive biomonitor of potential toxic effects (Brake and Evenson, 2004). Pregnant mice were fed a transgenic soybean or a non-transgenic (conventional) diet through gestation and lactation. After weaning, the young male mice were maintained on the respective diets. At 8, 16, 26, 32, 63, and 87 days after birth, three male mice and an adult reference mouse were killed, the testes surgically removed, and the cell populations measured by flow cytometry. Multigenerational studies were conducted in the same manner. In comparison with animals fed the conventional diet, no adverse effects on macromolecular synthesis or cell growth and differentiation were observed in mice given the transgenic soybeans. Moreover, no differences between groups were noted in litter size and body weights. The authors concluded that the transgenic soybeans did not cause negative effects on fetal, postnatal, pubertal or adult testicular development, or body growth in the mouse. Zhu et al. (2004) did not find adverse effects of glyphosate-tolerant soybean meal in rats at levels as high as 90% of the diet.

Any of the above studies reported results concerning potential endocrine effects of the GM soybeans. Information about it, as well as on the composition of GM soybeans is important taking into account that this crop has been used for preparation of soymilk and other products recommended as health food. With respect to the composition of GM soybeans, Cromwell et al. (2002) showed that Roundup Ready soybean meal was essentially equivalent in composition and nutritional value to conventional soybean meal for growing-finishing pigs. In turn, McCann et al. (2005) concluded that the composition of commercial glyphosate-tolerant soybeans over 3 years of breeding into multiple varieties remained equivalent to that of conventional soybeans. On the other hand, according to Kim et al. (2006) the allergenicity of wild type and GM soybeans extracts was identical in adults. However, other authors concluded that to assess the allergenicity of GM soybean and other GM food, more research, including a selection of controlled sample materials and immunoassays of qualified sera, is needed (Yum et al., 2005; Cantani, 2006).

Rice

Wang et al. (2000) investigated the safety of the anti-herbicide gene(BAR) transgenic rice. Acute toxicity studies, mutation tests and a 30-day feeding study were conducted in rats and mice. The oral LD_{50} in both species of mammals was >21.5 g/kg of body weight, while no mutations were found. Rats consuming 16.3 and 64 g/kg of body weight had a normal growth and development at the 30-day feeding test. Neither adverse effects on body weight nor histopathological alterations were noted.

Momma et al. (1999) showed that accompanying the higher protein level in GM rice with the soybean glycin gene, the contents of almost all amino acids including lysine were higher (20% more) in the GM rice. The high-level expression of the desired proteins had the possibility to provoke not only nutritional changes but also metabolic disturbances in the host crops. Therefore, the authors remarked that the safety assessment based on "substantial equivalence" would not be always enough to apply to the safety assessment of GM crops thus created. Thus, in

order to assess the effects of these metabolic fluctuations, this research group conducted in rats feeding studies on rice genetically modified with soybean glycin for four weeks. The administered amount was 10 g/kg-rat/day, which is ten times higher than that prescribed for the safety assessment of food additives. During the experimental period, no differences were noted in appearance, food intake, body weight, and cumulative body weight gain. There were also no significant differences in the blood count, or in the biochemical parameters determined in plasma. No abnormalities of organs were observed regarding weight, shape and function (Momma et al., 2000). In spite of these results, the authors concluded that the potential risks of unknown toxins in the GM rice, and the capability to induce malformations, reproductive disorders, mutagenicity, and carcinogenicity of the GM rice could not be confirmed by this short-term experiment (Momma et al., 2000). We absolutely agree with this conclusion, as most studies on potential health risks of transgenic foods are only short-term studies. In a subsequent investigation of the same research group, no biochemical, nutritional, or morphological abnormalities were detected in long-term chronic toxicity experiments (Momma et al., 2002). However, to date data on the ability of GM rice to induce mutagenicity, teratogenicity, and carcinogenicity are not available from the scientific literature.

A research group of the Institute of Nutrition and Food Safety of Beijing (China) recently reported a series of studies to assess in rodents the potential adverse effects of GM rice, which expressed insecticidal protein CpTI (cowpea trypsin inhibitor). Despite the evident scientific interest of these investigations, the results were only published in Chinese. One of these studies investigated if the transgenic rice possessed potential teratogenicity in weanling rats. Animals were divided into four groups: transgenic rice group, non-transgenic rice group, and negative and positive control groups. The diet of the non-transgenic rice group contained 74.7% of non-transgenic rice, which was the parent line of the transgenic one. When the sexual maturation period of rats arrived, conventional teratogenicity tests were performed. Body weight of pregnant rats, and body weight, body length, and tail length of fetuses were significantly higher in the transgenic rice group than in the positive control group, whereas the malformation rate of fetuses was significantly lower in the transgenic rice group. The transgenic rice modified with CpTI was considered to have neither maternal toxicity nor embryotoxicity/teratogenicity (Zhuo et al., 2004a). In turn, Li et al. (2004a) evaluated the effects of genetically modified rice with Xa21 on the development of rat embryos. Weanling rats were divided into four groups: transgenic rice group, non-transgenic rice group, AIN93G negative control group, and MATDA positive control group. The rats were fed with corresponding food for 90 days and mated. The development of maternal rats and embryos was observed. Body weight gain of pregnant rats, as well as body weight, body length, and tail length of fetuses in the transgenic rice group were significantly increased in comparison with those in the positive control group. The number of deaths and reabsorbed embryos, and the malformation rates (external, visceral, and skeletal) were lower in the transgenic rice groups than in the positive control group. Compared with the non-transgenic rice, transgenic rice modified with Xa21 gene did not show significant differences in rat pregnancy rate and embryo development.

The nutrition effects between transgenic and non-transgenic rice were also investigated in rats. Following 28 days of exposure, with the exception of the liver weight/body weight ratio, which in male rats was higher in the transgenic rice group than in the non-transgenic rice group, all other indicators did not show significant differences. In females, liver weight/body weight ratio, blood calcium and bone density were higher in the transgenic rice group than in the non-transgenic one. It was concluded that transgenic rice had good nutritional effects on rat development, while no adverse/toxic effects were observed in the transgenic rice group (Li et al., 2004b). It is important to note that the slight differences noted should not be underrated, especially taking into account that the experimental period was only 28 days. A semichronic study was also performed in weanling rats by the same research group (Zhuo et al., 2004b). Animals were divided into three groups: T, N, and C group. The diet of T group contained 78.3% of transgenic rice, while the diet of N group contained 74.7% of non-transgenic rice which was the parent line of transgenic one. The diet of C group was the standard diet AIN93G. Rats were fed for 90 days. In general, no significant nutritional differences among the three groups could be found, whereas no histopathological damage was noted. At the end of the first month, the male rats' body length of the T group was longer than that of the other two groups, while at the end of the test period, the male rats' blood glucose and ALT were lower than those in the other two groups. In the middle of the test period, the female rats' red blood cell number and hemoglobin were higher than those in the other two groups, while at the end of the test period, the female rats' monocyte number was higher than that found in the other two groups. However, all these results were in the normal range. Therefore, the authors concluded that the results of the 90 days feeding test of transgenic rice on rats did not reveal any signs of toxic and adverse effects. However, this was not a toxicological study, and therefore, the data are irrelevant from the toxicological point of view.

Recently, Schroder et al. (2006) reported the results of a 90-day safety study of GM rice (KMD1) expressing Cry1Ab protein (*Bacillus thuringiensis* toxin) in Wistar rats. The KMD1 rice contained 15 mg Bt toxin/kg. No adverse effects on animal behavior or weight gain were observed during the study. A few hematological and biochemical parameters were different from those considered as standard for Wistar rats, but all within the normal reference intervals for rats of this breed and age, and consequently not considered treatment related. Upon sacrifice, only minor changes were observed in a large number of organs on weight, macroscopic, and histopathological examinations. In spite of these results, Schroder et al. (2006) concluded that the safety assessment for unintended effects of a GM crop could not be done without additional test group(s).

To assess the potential immunotoxicologic effects of transgenic rice, a short-term feeding study was conducted in mice

(Chen et al., 2004). Animals were fed with food composed by transgenic rice (into which cowpea trypsin inhibitor gene was introduced) or non-transgenic rice (which had the same gene composition as the transgenic rice except for the cowpea trypsin inhibitor gene) for 30 days. At the end of this period, immunotoxicologic indexes of each group were compared (body weight, guts index, blood routine test, lymphocyte sort, serum antibody titter, plaque forming cell, delayed hypersensitivity response, and macrophage function test). No significant differences between transgenic rice and non-transgenic rice groups were observed. It was concluded that transgenic rice was substantially equivalent to non-transgenic rice in relation to immunotoxicologic effects.

Cucumber

Kosieradzka et al. (2001) examined in rats the effects of feeding diets with a considerable proportion of transgenic cucumber on growth parameters, relative organ weights, and nutrient digestibility. These effects were compared with those of feeding the fruits in balanced diets. The genetic modification consisted of introducing the gene coding a sweet protein, thaumatin, and the marker gene of resistance to kanamycin. The experiment was conducted for 5 weeks on 3 groups of male rats with an initial mean body weight of 150 g. Isoprotein diets containing 0 or 15% lyophilized transgenic or non-transgenic cucumbers did not affect weight gain, apparent health status, or relative organ weights of animals. Protein digestibility was slightly but significantly lower (89.2 vs. 90%) in diets containing transgenic cucumbers than in those contained non-transgenic cucumbers, whereas digestibility of crude fiber was higher in the group given non-transgenic cucumbers (28.2% vs. 15%). In turn, digestibility of fat and N-free extractives did not differ. Consequently, consumption of transgenic cucumbers for 28 days did not affect the growth and health of rats, although it did slightly affect nutrient digestibility. We agree with the conclusion of the authors noting that the influence of feeding transgenic plants on animal organisms requires more thorough and longer studies.

Tomatoes and Sweet Pepper

Noteborn et al. (1995) assessed in weanling rats the safety of the *Bacillus thuringiensis* insecticidal Crystal Protein CRY1a(b) expressed in transgenic tomatoes. During 90 days, rats ate tomato-diets, which on average corresponded to 20 g of fresh tomatoes per day. Percent survivals, final body weights, and organ (liver, kidneys, testes) weights, as well as macroscopic and microscopic examination of organs and tissues did not reveal significant differences between consumption of GM tomatoes and the unmodified parent.

In the early 1990s, a coat protein gene (*cp*) from a cucumber mosaic virus (CMV) Chinese isolate was cloned (Hu et al., 1990) and a genetic transformation system was established for sweet pepper and tomato plants. In order to assess the safety of GM sweet pepper and tomato with CMV-*cp* gene as food, Chen et al. (2003) conducted the following tests in rats and mice: acute toxicity assay, micronucleus test, sperm aberration test, Ames test, and 30-day animal feeding study. The LD_{50} for the two GM products was considered to be greater than 10 g/kg for rats and mice, indicating that liophylized GM powders were as innocuous as their non-GM counterparts. No genotoxicity either in vitro or in vivo by the micronucleus test, sperm aberration test, and Ames test were detected. Animal feeding studies did not show significant differences in growth, body weight gain, food consumption, hematology, blood biochemical indices, organ weights, and histopathology between rats or mice of either sex fed with either GM sweet pepper or tomato diets compared with those given non-GM diets. According to the authors, these results demonstrated that the CMV-resistant sweet pepper and tomato would be comparable to the non-GM counterparts in terms of food safety.

Peas

Pusztai et al. (1999) evaluated the effect of expression of bean alpha-amylase inhibitor (alpha-AI) transgene on the nutritional value of peas in pair-feeding rats diets (10 days) containing transgenic or parent peas at 300 and 650 g peas/kg, respectively, and at 150 g protein/kg diet, supplemented with essential amino acids to target requirements. The results were also compared with the effects of diets containing lactalbumin, with or without 0.9 or 2.0 mg bean alpha-AI, levels equivalent to those in transgenic pea diets. The weight gain and tissue weights of rats fed either of the two pea diets were not significantly different from each other or from those of rats given the lactalbumin diet even when this was supplemented with 0.9 g alpha-AI/kg. The digestibilities of protein and dry matter of the pea diets was slightly, but significantly lower than that of the lactalbumin diet. The nutritional value of diets containing peas at the higher (650 g) inclusion level was less than that of the lactalbumin diet. However, the differences between transgenic and parent pea lines were small, possibly because neither the purified recombinant alpha-AI nor that in transgenic peas inhibited starch digestion in the rat small intestine in vivo to the same extent as did bean alpha-AI. In conclusion, this short-term study indicated that transgenic peas expressing bean alpha-AI gene could be used in rat diets at 300 g/kg level without major harmful effects on their growth, metabolism and health, raising the possibility that transgenic peas might also be used at this level in the diet of farm animals. However, the authors remarked that at that stage, the results of their nutritional study could not be taken as a proof that transgenic peas were fit for human consumption. More specific risk assessment testing procedures, which must be designed and developed with human consumers in mind, would be clearly necessary. To date, and according to the literature, these studies have not been conducted yet.

Canola Plants

To evaluate the potential toxicity and allergenicity of green fluorescent protein (GFP), Richards et al. (2003) fed pure GFP and diets containing transgenic canola plants expressing GFP to weaned male rats for 26 days. GFP has become a valuable tool in biotechnology because it has unparalleled effectiveness as a real-time marker of promoter activity and gene expression in vivo. Animals were fed either AIN-93G (control), control diet plus 1.0 mg of purified GFP daily, modified control diet with 200 g/kg canola (*Brassica rapa* cv Westar), or control diet with 200 g/kg transgenic canola containing one of two levels of GFP. Ingestion of GFP did not affect growth, food intake, relative weight of intestine or other organs, or activities of hepatic enzymes in serum. A comparison of the amino acid sequence of GFP to known food allergens revealed that the greatest number of consecutive amino acid matches between GFP and any food allergen was four, suggesting the absence of common allergen epitopes. Moreover, GFP was rapidly degraded during simulated gastric digestion. These data indicated that GFP had a low allergenicity risk and provided preliminary indications that GFP would represent a minimal risk for the food supply. However, in their conclusions the authors remarked that this short-term study was not sufficient to guarantee the lack of potential health risks, and consequently, long-term feeding studies were required. These data are not currently available from the scientific literature.

GENETICALLY MODIFIED DNA IN FOOD

Humans typically consume a minimum of 0.1 to 1 g/day of DNA in their diet (Doerfler, 2000). Therefore, the transgene in a genetically engineered plant is not a new type of material to our digestive system, and it is present in extremely small amounts. There is no compelling evidence for the incorporation and expression of plant-derived DNA, whether as transgene or not, into the genomes of consuming organisms (SOT, 2003). Although much remains to be learned about the fate of dietary DNA in the mammalian systems, the possibility of adverse effects arising from the presence of transgenic DNA in foods, either by direct toxicity or gene transfer, would be minimal according to the WHO (2002) and other international regulatory organisms. Jonas et al. (2001) reviewed whether the consumption of DNA in approved novel foods and novel food ingredients derived from genetically modified organisms (GMOs) could be regarded as safe as the consumption of DNA in existing foods. It was concluded that the probability of transfer and functional integration of DNA from ingested food by gut microflora and/or human cells was minimal.

However, not all the investigators are in agreement with these conclusions. For example, the same WHO indicates that gene transfer from GM foods to cells of the body or to bacteria in the gastrointestinal tract would cause concern if the transferred genetic material adversely affects human health, which would be particularly relevant if antibiotic resistance genes, used in creating GMOs, were to be transferred (WHO, 2002). Although intact foreign DNA is not thought to be available for transfer into human cells, there is a remote possibility that DNA fragments may be taken up by bacteria in the gut (Donaldson and May, 1999). DNA fragments, after passing through the intestinal wall, might be actively removed by cells of the gut immune system or they might enter the circulation (Jonas et al., 2001). In relation to this, Schubbert et al. (1997) demonstrated that food-ingested foreign DNA was not completely degraded in the gastrointestinal tract of mice. Orally administered M13mp18 DNA could be recloned from spleen DNA in linkage to DNA with 70% homology to the mouse IgE receptor, whereas the DNA recloned from spleen also contained bacterial DNA possibly transported from the gut through the intestinal wall by a route akin to M13mp18 test DNA. In summary, foreign DNA ingested by mice might reach peripheral leucocytes, spleen, and liver via the intestinal-wall mucosa (Schubbert et al., 1997). Therefore, a gene that has been transferred might be incorporated in an unpredictable place in the genome (Godfrey, 2000). In the UK, a report on the health implications of GM foods concluded that "there is no current evidence that GM technologies used to produce food are inherently harmful; this is true, but one cannot conclude that all application will be harmless" (htpp://www.doh.gov.uk/gmfood.htm).

The results of a study on the implications for the possible transfer of genes from GM food (Chiter et al., 2000) raised also some uncertainties. It was demonstrated that the treatment of plant tissues at temperatures of 95°C or above for more than a few minutes was sufficient for degradation of DNA to take place to the extent that it should be incapable of transmitting genetic information. However, materials that had not been subjected to such treatments not only had non-fragmented DNA but also retained specific polymerase chain reaction (PCR)-detectable sequences suggesting that DNA was intact. It would imply that stringent conditions are needed in the processing of GM plants for food consumed by animals and humans to eliminate the possibility of transmission of transgenes. Similar conclusions were also drawn by Chowdhury et al. (2003), who tried to detect maize DNA fragments in the intestinal contents of pigs fed GM maize (atarlink CBH351) or non-GM maize by PCR. These authors suggested that ingested DNA was not totally degraded, but rather was present in a form detectable by PCR.

On the other hand, Duggan et al. (2003) using the PCR technique, investigated the fate of a transgene in the rumen of sheep fed silage and maize grains from an insect-resistant maize line. Free DNA survived in a functional state for a significant amount of time in the ovine oral cavity, suggesting that DNA released from the diet might transform competent oral bacteria. By contrast, the chances of microbial transformation in the rumen and lower regions of the ovine digestive system would be likely low due to a high level of nuclease activity. Nevertheless, a rare transformation event would be significant if the donor DNA is an antibiotic resistance gene and the recipient is a human or animal pathogen. The authors concluded suggesting that the use of GM crops harboring antibiotic resistance genes, in particular

the use of unprocessed grains in animal feed, deserved further evaluations.

In their investigations on GM maize (Bt-maize) in pig nutrition, Reuter and Aulrich (2003) also showed that feed-ingested DNA was partially resistant to the mechanical and enzymatic activities of the gastrointestinal tract and was not completely degraded. Small DNA fragments derived from feedstuff could pass the gut wall and might enter organs and tissues of pigs.

CONCLUSIONS

In recent years, three reviews on similar topics than that of the current paper have been published. Zdunczyk (2001) concluded indicating that for a safe use of transgenic food, evaluation of the concordance of the chemical composition of transgenic and conventional crops ("substantial equivalence") would not be sufficient. Subchronic in vivo studies, as well as a comparison of the nutritional equivalence of transgenic and conventional crops are advisable. These actions would be justified not only by the possibility of undesirable transgenic effects, but also by the consumer's right to explicit information on food safety.

In a wide review of the scientific literature on the potential adverse health effects of genetically modified crops, Bakshi (2003) indicated that these were generally safe their consumption being not associated with serious health problems. However, this author remarked that because genetic engineering of crops was a new technology in its embryonic stages, scientists still had an incomplete understanding of physiology, genetics, and nutritional value of genetically engineered crops. It leads to the inability to predict everything that can go wrong, including many risks that have not been identified. Some concerns are that GM crops may contain allergenic substances due to the introduction of new genes into crops, or that genetic engineering often involves the use of antibiotic-resistance genes as "selectable markers," which could lead to production of antibiotic-resistant bacterial strains that are resistant to available antibiotics. The genetically modified crops might contain other toxic substances (such as enhanced amounts of heavy metals) and the crops might not be "substantially equivalent" in genome, proteome, and metabolome compared with unmodified crops.

Pryme and Lembcke (2003) reviewed literature published in vivo studies on possible health consequences of genetically modified food and feed where the ingredients in question consisted of genetically modified plant materials. According to a Norwegian report "Gen-mat" (NOU 2000:29), and a more recent search in Medline and Citations Index, they only found a total of ten studies on the health effects of GM-foods and feeds. The authors concluded that much more scientific effort and investigation would be necessary before guaranteeing that eating foods containing GM material in the long-term will not be a probable cause of health problems. They considered essential to test in a transparent manner each individual GM product before its introduction into the market.

The conclusions of the current review are quite in agreement with those of Zdunczyk (2001), Bakshi (2003), and Pryme and Lembcke (2003), which are in the same line than those also suggested in our previous review (Domingo and Gómez, 2000). One of our main concerns is related with the use of the principle of "substantial equivalence" to guarantee the safe use of GM/transgenic plants. Why must it be thought that two plants (GM and non-GM) with the same nutritional capacity should also imply similar health risks (or absence of risks)? Why a similar principle is not authorized, for example, for chemical substances that are going to be commercialized such as pesticides, drugs, food additives, etc.? It is currently admitted that this principle is a starting point rather than an end point. If this seems to be quite clear, why the published information is so scant, taking into account that the debate about the safety of GM plants generates a great controversy?

In summary, the above seems to indicate that regulatory agencies reduce the concern for human health risks derived from the potential tendency to provoke gene transfer following consumption of GM foods. However, experimental studies carried out by independent researchers do not underrate the possibility that a transgene could be itself toxic of be transferred to the genome of the consumer. Recent investigations have concluded suggesting the necessity of further investigations on this important issue. With respect to this, in 1999, Ewen and Pusztai emphasized two potentially relevant concerns:

(1) the scant attention that has been given to people with abnormal digestion as a result of chronic gastrointestinal disease, and
(2) the possibility of allowing unexpected enhancement of intercurrent viral infection, taking into account the widespread mucosal accessibility to food viral DNA, a hot spot of DNA recombination.

Similarly, in countries where HIV-1 infection is endemic, the assumption that a viral component of GM food is harmless might be misplaced.

The main goal of the present paper has been to review critically the published scientific literature concerning potential toxic effects/health risks of GM plants. It has been noted that experimental data are very scarce. As shown throughout the paper, most investigations correspond to short-term studies, mainly nutritional studies, with very limited toxicological information (Filip et al., 2004). Where are long-term toxicological studies that should guarantee the safety of the transgenic plants for animal and human consumption? (Patel et al., 2005). Because of the importance that the consumption of GM foods has acquired, as well as its enormous potential in the near future, the performance of a complete case-by-case study seems would be advisable (Weil, 2005). Long-term studies are clearly necessary. This review can be concluded raising the following question: where is the scientific evidence showing that GM plants/food are toxicologically safe, as assumed by the biotechnology companies involved in commercial GM foods?

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Health Risks of Genetically Modified Foods

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As genetically modified (GM) foods are starting to intrude in our diet concerns have been expressed regarding GM food safety. These concerns as well as the limitations of the procedures followed in the evaluation of their safety are presented. Animal toxicity studies with certain GM foods have shown that they may toxically affect several organs and systems. The review of these studies should not be conducted separately for each GM food, but according to the effects exerted on certain organs it may help us create a better picture of the possible health effects on human beings. The results of most studies with GM foods indicate that they may cause some common toxic effects such as hepatic, pancreatic, renal, or reproductive effects and may alter the hematological, biochemical, and immunologic parameters. However, many years of research with animals and clinical trials are required for this assessment. The use of recombinant GH or its expression in animals should be re-examined since it has been shown that it increases IGF-1 which may promote cancer.

Keywords Allergenicity, antibiotic resistance, food safety, genetically modified, health risks, recombinant growth hormone, toxicity

INTRODUCTION

Nearly fifteen years have passed after the introduction of genetic modifications (GM) in food and new GM food are added in the existing list of foods. Who could imagine that there would come a day when the pig would be as "fat –free healthy food" as a fish or that the ice cream our children eat would contain a protein from the fish? Are GM safe to human health? Studies concerning their safety are still few when one considers the toxicity studies that must accompany the application of any novel drug for approval by the corresponding drug administration. The results from most toxicity studies available in literature are reviewed and the significance of these findings is discussed. In the absence of adequate safety studies, the lack of evidence that GM food is unsafe cannot be interpreted as proof that it is safe. Furthermore, if they are not considered safe for human consumption why should they be approved for animals? Humans can inadvertently consume foods that contain GM products fed to animals, i.e., crops modified for enhanced productivity in animals. This was the case when traces of a StarLink GM crop, restricted for use only in feed, were found in taco shells already in the market. One has to wonder what will happen if we start consuming food crops contaminated with GM crops containing genes for the production of drugs and industrial chemicals that have never been assessed for their toxicity? (Margulis, 2006). The debate over its safety continues. One should not forget that every single GM food through the food chain will eventually reach the consumer. Issues such as the concern of the public for possible hazards due to the consumption of a GM food have already been discussed, but there is always something to add. However, prior to discussing these issues one must take into account in brief the regulation of testing for GM food safety.

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THE STANDARDS AND REGULATION OF TESTING FOR FOOD SAFETY

In Europe, the placing on the market of genetically modified foods is covered by Regulation (EC) 1829/2003 on genetically modified food and feed. Multiple guidelines for the safety assessment process of GM foods have been developed (FAO/WHO, 2000; EFSA, 2005) and the new approach designed by ENTANSFOOD to guide the choice of test methods for this safety

assessment requires compositional analyses of key nutrients and anti-nutrients in GM crops (Kuiper et al., 2004).

Another issue of great importance for the EU consumer following the carried out consumer studies is the GM (novel food) area which lead to a confrontation between USA and EU. A novel food is defined as a food or food ingredient which does not have a significant history of consumption within the EU prior to May 1997. All novel foods are subject to a pre-market safety assessment under the Novel Foods Regulation (EC) No. 258/97. The Food Standards Agency Board is satisfied that the current safety assessment procedures for GM foods are sufficiently robust and rigorous to ensure that approved GM foods are as safe as their non-GM counterparts, and pose no additional risk to the consumer. Each GM food is assessed for safety, including its toxicological, nutritional, and allergenic potential, on a case by case basis before it can be approved for marketing (Arvanitoyannis et al., 2005). The EU and US legislation focused on GMOs is given in Table 1.

The cultivation of new GM crop events also remains far on the horizon in the EU. On 7 December 2005, the EFSA adopted a first positive opinion for cultivation of the GM potato event EH92-527-1. However, its cultivation will be restricted to a closed loop system of contractors (EFSA, 2006). Moreover, the European adoption rate of previously approved GM crops for cultivation was slow (Demont and Tollens, 2004). With the registration of seventeen MON810 hybrids in the common seed catalogue on 8 September 2004, the GM maize cultivation area increased in France, Germany, and Spain, and expanded to the Czech Republic and Portugal in 2005. Nonetheless, in 2005, the European cultivation area of GM maize was approximately 55000 ha, whilst globally 21.2 million ha was reached (Devos et al., 2006).

The results are evaluated based on the principle of "substantial equivalence" criticized by Millstone et al. (1999) as "being created to provide an excuse for not requiring biochemical and toxicological tests." Moreover, Burlingame (2004) states that existing food composition databases do not necessarily reflect the complete natural variation since it was shown that the protein content may be different for both the transgenic and the parental line. Although genomics, proteomics, and metabolomics will provide a "global" overview of gene expression and have the potential for generating massive amounts of data, the possibility of predicting toxicity would still remain low due to complex metabolic pathways (Cellini, 2004). Taking into consideration the possibility that an analytical method might give false negative results for a toxic substance that may be produced in a GM food, this principle should not be the limiting step in evaluating GM crop safety. "Substantial equivalence" may provide some theoretical points background in predicting toxicity, but in practice the only reliable way to evaluate the toxicity of a GM food is through toxicity tests on animals. Furthermore, it has been argued that GM foods should be subjected to the same testing and approval procedures as medicines (i.e., clinical trials) since they must be adequate to ensure that any possibility of an adverse effect on human health from a GM food can be detected.

HAZARDS OF GM FOOD

Possible hazards of GM food for animals and populations exposed to a diet containing GM products include the potential for pleiotropic and insertional effects, effects on animal and human health resulting from the increase of anti-nutrients, potential effects on human health resulting from the use of viral DNA in plants, possible transfer of antibiotic resistant genes to bacteria in gastrointestinal tract, and possible effects of GM foods on allergic responses.

The Potential For Pleiotropic and Insertional Effects

Concern has been expressed about the above potential effects which might cause the silencing of genes, changes in their level of expression or, potentially, the turning on of existing genes that were not previously being expressed (Conner and Jacobs, 1999). This interaction with the activity of the existing genes and biochemical pathways of plants, may lead to disruption of metabolism in unpredictable ways and to the development of new toxic compounds or an increase of the already existing ones as it happened with two genetically produced foods, tryptophan and g-linolenic acid (Hill et al., 1993; Sayanova et al., 1997). Moreover, research into epigenetics has also revealed that genes account for only a part of the control of the biochemistry of organisms, and organisms have a level of control above genes that interact with genes explaining why genetic engineering is so unpredictable, with different results produced by each attempt and why the products are often unstable. The possibility that an unidentified compound may be present in the GM food makes crucial that each transgenic food as whole food and not as a single protein should be tested directly for toxicity in animals, although as Kuiper et al. (2004) state there are limitations in establishing dose-response relationships.

Possible Effects on Animal Health Resulting from the Increase of Anti-nutrients

The insertion of a new gene can sometimes lead to increase in existing levels of anti-nutrients, some of which cannot be reduced with heat treatment (Bakke-McKellep et al., 2007). One of the most widely available commercial GM products nowadays glyphosate-resistant Roundup Ready® soybean may display an increase in anti-nutrients (Padgette et al., 1996). Heat-stable anti-nutrients such as phytoestrogens, glucinins, and phytic acid were also found to cause infertility problems in sheep and cattle (Liener, 1994), allergenic reactions and binding to phosphorus and zinc thereby making them unavailable to the animal respectively (Adams, 1995). An increase in the anti-nutrient level should not be accepted since a GM food may be consumed as raw material.

Table 1 EU Directives and Regulations and US Acts (main points and comments) for GMOs

Title	Main points	Comments
	EU legislation	
Directive 90/219/EEC (entry into force 23/10/1991) Contained use of G.M. Microorganisms	▪ Measures for limited use of GM micro-organisms. ▪ Not applicable to certain techniques of genetic modification. ▪ Measures for avoidance of adverse effects in human health and environment.	➢ Directive 98/81/EC amended this Directive (entry into force 5/12/1998)
Directive 90/220/EEC (entry into force 23/10/1991) Deliberate release into the environment of GMOs	▪ Protective measures for human health and environment. ▪ Not applicable to certain techniques of genetic modification. ▪ Activities of Member States for deliberate release into the environment of GMOs for research, development and market placing purposes.	➢ Directive 97/35/ECAnd Regulations (EC) No.258/97 and No.1139/98 amended this Directive
Directive 2001/18/EC (entry into force 17/4/2001) Deliberate release into the environment of GMOs	▪ Measures of authorization of the release and disposal on the market of GMOs. ▪ Obligatory controls after the disposal of GMOs on the market. ▪ Consultations with the public and labelling of GMOs.	➢ The last amendment of this Regulation (EC) No.1830/2003 (entry into force 7/11/2003)
Directive 2004/204/EC (entry into force 23/3/2004) Arrangements for the operation of the registers for recording information on genetic modifications in GMOs	▪ Lists of information of genetic modification in GMOs. ▪ Lists should contain detailed report of documents. ▪ Lists are public available.	
Directive 2004/643/EC Placing on the market of a maize product (*Zea mays* L. line NK603) GM for glyphosate tolerance	▪ Product should be as safe as conventional (equivalence principle). ▪ Obligatory recordation of the code MON-00603-6 (unique). ▪ Measures for labelling and traceability in all stages of the market promotion.	
Directive 2004/657/EC Placing on the market of a sweet corn from GM maize line Bt11 as a novel food or novel food ingredient	▪ Product should be as safe as conventional. ▪ Obligatory labelling as “GM sweet corn.” ▪ Obligatory recordation of the code SYN-BTø11-1 (unique).	
Regulation (EC) No.258/97 (entry into force 14/5/1997) Novel food and novel food ingredients	▪ Placing on the market within the Community of foods and food ingredients which have not been used for human consumption to a significant degree within the Community before. ▪ Not applicable to food additives, flavourings and extraction solvents. ▪ Specific procedure for foodstuffs containing GMOs.	
Regulation (EC) No.1139/98 (entry into force 1/9/1998) The compulsory indication of the labelling of certain foodstuffs produced from GMOs	▪ Application to food and food ingredients which are produced from GM soybean or GM corn. ▪ No application to food additives and condiments. ▪ No application to products which are legally produced, labelled and imported, commercialized in the Community.	➢ Regulations (EC) No.49/2000 and No.50/2000 amended this one.
Regulation (EC) No.1829/2003 (entry into force 7/11/2003) GM food and feed	▪ Measures for human and animal health protection, Community procedures of approval, inspection and labelling of GM food and feed. ▪ Approvals are applicable for 10 years with the potential of renewal.	
Regulation (EC) No.1830/2003 (entry into force 7/11/2003) Traceability and labelling of GMOs and traceability of food and feed products produced from GMOs	▪ Traceability of products consisting of, or containing GMOs and foodstuffs, feed produced from GMOs. ▪ Application for all stages of disposal on the market. ▪ Specific demands on labelling.	
Regulation (EC) No.65/2004 (entry into force on the date of its publication in the *Official Journal of the European Union*) Establishment of a system for the development and assignment of unique identifiers for GMOs	▪ Unique identifier for each GMO which is placed on the market. ▪ Not applicable to pharmaceuticals intended for human and veterinary use.	
Regulation (EC) No.641/2004 (entry into force 18/4/2004) The authorization of new GM food and feed, the notification of existing products and adventitious or technically unavoidable presence of GM material which has benefited from a favorable risk evaluation	▪ Transformation of applications and statements in the applications. ▪ Requirements of input on the market of certain products. ▪ Transitional measures for adventitious or technically unavoidable presence of GM material which has benefited from a favorable risk evaluation.	
Proposal for a Regulation COM/2002/0085 – COD 2002/0046 (entry into force 27/10/2002) The transboundary movement of GMOs	▪ Establishment of a notifying system and exchanging information on the exports of GMO to third countries. ▪ No application for pharmaceuticals for human use. ▪ Surveillance, submission of reports, and imposition of sanctions for any infringement.	

(Continued on next page)

Table 1 EU Directives and Regulations and US Acts (main points and comments) for GMOs *(Continued)*

Title	Main points	Comments
	US legislation	
Genetically Engineered Food Safety Act, 2003	▪ Definitions (genetically engineered organism, genetically engineered material etc) ▪ Federal determination of safety of genetically engineered food, regulation as food additive ▪ Rulemaking, effective date, previously unregulated marketed additives	
Genetically Engineered Crop and Animal Farmer Protection Act, 2003	▪ Definitions (genetically engineered plant, genetically engineered animal, genetically engineered material etc.) ▪ Contract limitations regarding sale of genetically engineered seeds, plants, and animals ▪ Prohibition on labelling certain seeds as non-genetically engineered	
Genetically Engineered Food Right to Know Act, 2003	▪ Definitions (genetically engineered organism, genetically engineered material etc.) ▪ Requirements for labelling regarding genetically engineered material ▪ Misbranding of food with respect to genetically engineered material	
Genetically Engineered Pharmaceutical and Industrial Crop Safety Act, 2003	▪ A pharmaceutical crop or industrial crop is a plant that has been genetically engineered to produce a medical or industrial product, including a human or veterinary drug, biologic, industrial, research chemical, or enzyme. ▪ Definitions (genetically engineered plant, genetically engineered animal, genetically engineered material etc.) ▪ Report to Congress on alternative methods to produce pharmaceutical and industrial crops	

Potential Effects on Human Health resulting from the use of Viral DNA in Plants

Most of the manipulated crops utilize the Cauliflower Mosaic Virus 35S promoter (CaMV35S) to switch on the introduced gene. There has been a lot of controversy concerning whether the highly infectious CaMV35S can be horizontally transferred and cause disease, carcinogenesis, mutagenesis, reactivation of dormant viruses and even generation of new viruses (Hodgson, 2000). According to Ho et al. (2000), CaMV found in normal foods is not highly-infectious and cannot be absorbed by mammals. In contrast others believe that although humans have been ingesting CaMV and its 35 s promoter at high levels it has never been shown to cause disease in humans or to recombine with human viruses (Paparini and Romano-Spica, 2004). The transient expression in mammalian cells of transgenes transcribed from the CaMV35S promoter reported by Tepfer et al. (2004) raised the possibility that genes controlled by the 35S promoter have the potential for expression in animals. On the contrary, in recent studies Paparini and Romano-Spica (2006) failed to detect DNA transfer in mice and CaMV35S transcriptional activity with real time polymerase chain reaction (PCR), although they do emphasize the need for further studies.

Possible Transfer of Antibiotic Resistant Genes to Bacteria in the Gastrointestinal Tract

An area of concern focuses on the possibility that antibiotic resistance genes used as markers in transgenic crops may be horizontally transferred to pathogenic gut bacteria, thereby reducing the effectiveness of antimicrobial therapy. Although this probability is considered to be low (Halford and Shewry, 2000) other marker genes, such as the jellyfish green fluorescent protein (GFP) gene have been utilized. The only study assessing toxicity and allergenicity of GFP in male rats for 26 d, concluded that GFP exhibits a low allergenicity risk (Richards et al., 2003). It should be emphasized that only one transgenic plant (canola) containing GFP has been tested for toxicity. Every transgenic organism containing a new marker gene should be tested for toxicity with long term studies, since GM food will be consumed for a life time.

Possible Absorption of Genes Introduced in a GM Plant from the Gut

One concern associated with GM foods is the possibility that genes introduced into the plant might be taken up by the gut and become incorporated into the genetic make-up of consumers. In recent studies, Jennings et al. (2003 and 2003b) failed to detect fragments of the glyphosate resistant in a variety of tissue samples from pigs, fed glyphosate-tolerant soybeans and of transgenic and endogenous plant DNA in the chicken breast muscle. These findings are in contrast with those of Schubert et al. (1994), who reported that orally administered naked M13 phage DNA was detected in the mice blood. Moreover, short DNA fragments of GM plants have been detected in white blood cells and in milk of cows and in chicken and mice tissues that had been fed GM corn and soybean, respectively (Beever and

Kemp, 2000; Einspainer et al., 2001; Hohlweg and Doerfler, 2001; Phipps and Beever, 2001). Furthermore, fragments of recombinant cry1Ab gene were detected in the gastrointestinal tract of *Bacillus thuringiensis* (Bt)11 corn-fed pigs but not in the blood (Chowdhury et al., 2003). Therefore, it seems plausible that small amounts of ingested DNA are not broken down under physiological digestive processes. The fact that fragments of transgenic genes may not be detected in blood but can be detected in tissues of animals by PCR, underlies that they are in quite low levels in circulation and more sensitive methods of detection are needed (Puztai 2001). Moreover, Murray and his coworkers (2007) showed that not all PCR assays can detect DNA in extractions of shortly cooked corn, making the interpretation of the results from PCR even more difficult. These limitations in the detection of GM DNA should make us reconsider the view that gene transfer cannot occur, which falls in agreement with the findings of Netherwood et al. (2004) that transgene from GM soya survived passage through the small bowel in human ileostomists. According to Flachowsky (2005) the uptake of GM DNA into cells of the gastrointestinal tract will normally have no biological consequences because the DNA will be degraded in the cell. The question is whether it can be degraded in patients with severe gastrointestinal diseases. In the unlikely event that the DNA is recombined into a host chromosome, the probability that it will exert any biological effect on that cell remains unknown.

Possible Effects of GM Foods on Allergic Responses

The introduction of novel proteins into foods such as a GM soybean variety expressing methionine from Brazil nut (Nordlee et al., 1996) and GE corn variety modified to produce a Bt endotoxin, Cry9C (Bernstein et al., 2003) may elicit potentially harmful immunological responses, including allergic hypersensitivity (Conner et al., 2003; Taylor and Hefle, 2002). Moreover, according to Prescott et al. (2005) the introduction of a gene expressing nonallergenic protein such as GM field pea, expressing alpha-amylase inhibitor-1, may not always result in a product without allergenicity. This study underlines the need to evaluate new GM crops on a case-to-case basis and to improve the screening requirements for GM plants.

Brassica juncea, another GM plant, expressing choline oxidase gene caused low IgE response in mice and a cross-reactive epitope search showed a stretch similar to Hev b 6 having some antigenic properties although according to Singh et al. (2006) it had no allergenicity. These findings should be more carefully interpreted and repeated in other animal series in order to elucidate whether IgE response may play a role in toxicity.

As for Bt expressed in many crops, farm workers exposed to Bt pesticide may develop skin sensitization and IgG antibodies to the Bt spore extraction (Bernstein et al., 2003). "Antifreeze" protein which is produced through GM yeast, expressing a protein derived from fish is being considered for use in foods such as ice creams. Bearing in mind that allergy to fish is well established, a potential risk from such proteins to susceptible human beings exists although the only clinical study investigating this potential has shown that it does not possess allergenicity (Crevel et al., 2007).

Allergenicity Assessment

To evaluate allergenicity of GM foods the decision tree approach was developed in 1996 (Metcalfe et al., 1996) has been revised (FAO/WHO, 2001, Metcalfe, 2003). Risk assessment of the whole GM plant must consider whether allergenicity or toxicity of the crop could be increased. This is particularly important when the non-GM host plant is known as allergen or toxin source. Toxicity testing most often includes a 90-day toxicity study in rodents; allergenicity testing is done by comparison of the allergen repertoire of the GM crop with that of the conventional non-GM variety. Another aspect that is of concern when considering the extrapolation of the whole GM crop or food/feed toxicology and allergenicity studies carried out with single GM events to the GM stacked event, are the potential interactions of the newly introduced genes, regulatory sequences, and proteins (or its metabolites) with the host genome of the GM stacked event. Given that the transgenic DNA sequences/proteins are brought into a different genetic background, namely the stacked genetic background, their interaction with the genome might change, particularly if regulatory proteins, such as in experimental stress-resistant crops described in literature, are involved (De Schrijver et al., 2007).

Criticism on this approach includes the limited predictive ability of the amino acid sequence analysis for sequence similarity to known allergens (Alinorm, 2003; Prescott and Hogan, 2005). In vitro assessing degradability has also been questioned whether it can be correlated with allergenicity (Bannon et al., 2003) and instead Pusztai et al. (2003) proposed its replacement with in vivo (animal/human) testing. It has been emphasized that animal models used to assess the potential allergenicity of GM foods need to be validated. Studies with animals such as BALB/c mouse, HLA transgenic mouse, swine and atopic dog have shown that no single model can meet the requirements for an ideal model covering both the respiratory allergens as well as the gastrointestinal and dermatologic reactions (Tryphonas et al., 2003). Moreover, the model's ability to sensitize or alter endogenous protein expression may not be readily captured due to genetic differences across species (Germolec et al., 2003).

The questions in the area of human clinical data for the evaluation of protein allergenicity of GM foods have been discussed in detail (Germolec et al., 2003). Issues concerning human studies in individuals not only with an allergy history but with immunodeficiency problems as well should be included in a future discussion of the problem.

It has also been suggested that the oral consumption of a certain GM plant expressing a known allergen can help allergic individuals, since in rats GM lupine stimulates the development of a protective regulatory T-cell response and suppresses the development of allergic airways disease (Prescott and Hogan,

2005). One should consider whether this protective mechanism is stimulated in allergic immunodeficient patients. Moreover, it is not known whether the expression of an allergic reaction plays a protective role against other diseases that might have been caused by the exposure to this allergen.

POSSIBLE EFFECTS OF GM FOODS IN ANIMALS

Only recently a body of evidence is starting to emerge from a small number of animal feeding trials into the health effects. Ewen and Pusztai (1999) were the first to demonstrate the need to thoroughly test each GM plant product on animal models. The effects of most GM foods in animals are reviewed and include also the reanalysis of the controversial data reported by Monsanto's 90-Day feeding study on GM corn Mon863 (Seralini et al., 2007). As Varzakas et al. (2007) state, Member states should carefully scrutinize all applications, because companies try to hide information about the health impacts of GM. Although long –term feeding of high levels of individual "foods" to animals can result in nutritional imbalance (Varzakas et al., 2007) it should be stated that this is the only way that any substance can reveal its toxicity.

Effects on Growth

Body weight might be significantly altered as it has been shown with the consumption of Mon863 corn (Seralini et al., 2007) and GM rice on rats (Li et al., 2004).

Effects on the Gastrointestinal Tract

Stomach erosion and necrosis were reported in rats fed with flavr-savr™ GM tomatoes, while GM potatoes expressing Galanthus nivalis (GNA) lectin induced proliferative growth in their stomach which is of particular importance if one takes into consideration that glomelular stomach erosions can lead to life-threatening hemorrhage, especially in the elderly and patients on nonsteroidal anti-inflammatory agents (Pusztai et al., 2003). Intestines may also be affected by GM food consumption as it has already been shown with GM potatoes expressing Bt-toxin which caused the disruption, multinucleation, swelling, and increased degradation of ileal surface cells in rats (Fares and El-Sayed, 1998), GM potatoes expressing gna which induced proliferative growth in the small-large intestines (Ewen and Pusztai, 1999a) and GM soybean type Roundup Ready® which caused moderate inflammation in the distal intestine of salmons (Bakke-McKellep et al. 2007).

Recent work with gene transfer research has resulted in the production of the aquatic species with enhanced abilities in areas such as growth, cold tolerance, disease resistance, and metabolism of plant-based diets. Research with transgenic GHs has made the most progress, with the patented production of a line of Atlantic salmon capable of increased growth and feed conversion efficiency. This product has been licensed to a major biotechnology company and is currently awaiting regulatory approval for commercial use in the United States and Canada. Although transgenic research with invertebrates is far behind that for vertebrates, there is much potential for generic improvements among commercial bivalve species. Recent advances include development of successful, patented gene transfer methods, and research into boosting disease resistance. Despite the potential for GMOs in aquaculture, a number of environmental and human health concerns remain. Major concerns include escapement of transgenic fish into the wild, where they could disrupt natural gene pools through breeding with wild species, and the possible detrimental effects of introducing transgenics into the human and aquatic food chains (Rasmussen and Morrissey, 2007).

Binding to surface carbohydrates of the mouse jejunum was also revealed with Cry1Ac protoxin of the Cry genes, the most common terminators applied in currently approved crops (Vazquez-Padron et al., 2000). According to Pusztai et al. (2003) since it is the genetic manipulation process itself which led to toxicity, similar hazards might be seen in animals or humans fed genetically-manipulated soya, canola, and corn over a long period of time (i.e., years or decades). The chronic inflammation and proliferative effect that may be caused by some GM plants on the gastrointestinal tract may lead after years to cancer.

As for the effects of GM food on liver there are only a few long-term studies. It has been found that GM soya can alter the cell structure and functioning of the liver in mice reversibly (Malatesta et al., 2002; 2003; 2005) and can cause changes in histomorphology (Ostaszewska et al., 2005) and the protein profile of the liver in rainbow trout (Martin et al., 2003). Alterations have also been observed in hepatic enzymes after consumption of raw rice expressing GNA lectin (Poulsen et al., 2007), GM Bt with vegetative insecticidal protein gene (Peng et al., 2007) and in DuPont's subchronic feeding study in rats fed diets containing GM corn 1507 (MacKenzie et al., 2007). These alterations in hepatocyte cells and enzymes may be indicative of hepatocellular damage. Consumption of Mon863 corn in rats led to increase in trigycerides in females (Seralini et al., 2007).

Pancreatic Effects

GM soybean has also an impact on pancreas, since changes occurred in pancreatic acinar cells of mice and a high synthetic rate of zymogen granules containing low amounts of α-amylase (Malatesta et al., 2003).

Another target organ of some GM crops is the kidney. Smaller kidneys were developed in DuPont's study in rats fed diets containing GM corn 1507 (MacKenzie et al., 2007), whereas consumption of Mon863 corn in rats led to lower urine phosphorus and sodium excretion in male rats. There were also small increases in focal inflammation and tubular degenerative changes

characteristic of a classic chronic progressive nephropathy (Seralini et al., 2007). Rats fed GNA rice had elevated creatinine plasma concentration either due to some kind of renal effect or the increased water consumption in order to excrete the excess iron in the GNA rice diet (Poulsen et al., 2007). Salmons fed GM soybean had higher head kidney lysozyme and higher acid phosphatase activities (Bakke-McKellep et al., 2007).

Alterations in Hematology

Response variables were observed in animals fed with GM crops. DuPont's study in rats fed diets containing GM corn 1507 showed a decrease in red blood cell count and hematocrit of females (MacKenzie et al., 2007) while GM corn Mon863 affected the development of blood with fewer immature red blood cells (reticulocytes) and changes in blood chemistry in rats (Seralini et al., 2007). Bt with VIP insecticidal protein gene caused a decrease in platelets, monocytes ratio in female rats, and an increase in the granulocytes ratio in male rats (Peng et al., 2007).

As for the effects of GM crops on the immune system an increase in the production of Cry9C-specific IgG and IgG1 in rats and mice fed with GM heat-treated corn CBH351 was observed (Teshima et al., 2002) because the Cry gene possesses immunogenic properties as it was shown by Vazquez-Padron et al. (1999). Serum IgG mediates the inhibition of serum-facilitated allergen presentation. The presence of enhanced IgG Abs activates the IgG response (van Neerven et al., 1999) thereby indicating the occurrence of an allergic reaction having occurred, although Germolec et al. (2003) suggest that antigen specific IgG does not correlate to clinical allergy.

Moreover, GM corn Mon863 caused higher white blood cell levels in male rats (Seralini et al., 2007). DuPont's subchronic feeding study in rats fed diets containing GM corn 1507 showed that eosinophils concentration in females was decreased (MacKenzie et al., 2007). Rats given a diet based on GNA rice showed enlargement of the lymph nodes, and decreased weight of the mesenteric and of the female adrenal lymph nodes which may be indicative of an immune toxic response (Poulsen et al., 2007).

Effects on Biochemical Parameters

Subchronic feeding of GNA rice in rats resulted in decrease in glucose, while cholesterol, trigyceride, and HDLD concentration were higher (Poulsen et al., 2007).

Mortality

An increased mortality was observed in rats fed with GM tomatoes since seven out of forty rats died within two weeks without any explanation (Pusztai et al., 2003).

Reproductive and Developmental Toxicity

Of particular concern is the exposure of infants and children to GM foods because of their possible enhanced susceptibility for untoward effects. Only a limited number of studies regarding this topic are available, quite a few studies concerning this subject exist. Food-ingested M13 DNA fed to pregnant mice, was detected in various organs of fetuses and newborn animals, suggesting a possible transfer through the transplacental route (Doerfler and Schubbert, 1998). Maternally ingested foreign DNA could be a potential mutagen for the developing fetus.

Birthrates of piglets fed GM corn in Iowa country displayed an 80% fall due to high levels of Fusarium mold (Strieber, 2002), although it has been claimed that Bt corn expressing Cry proteins is less contaminated with mycotoxins (Weil, 2005). A Russian rat study reported very high death rates in the young of rats fed GM soya (56% died) in stunted growth in the surviving progeny (Ermakova, 2005). A study of GM rice expressing Xa21 on the development of rat embryos showed that there was an increase in the body weight gain of pregnant rats, the body weight, body length, and tail length of fetal rats (Li et al., 2004) whereas GM rice expressing cowpea trypsin inhibitor caused an increase in the male rats' body length and in the female rats' red blood cell number, hemoglobin, and monocyte number (Zhuo et al., 2004). The fact that no adverse effects have been observed in a reproductive and developmental study of bar gene inserted into GM potato may be due to the very low content of GM potato in food, so that the undesired effects are masked (Rhee et al., 2005).

GM food should be assessed for unexpected health effects in a vulnerable population such as children since after the first year their consumption is inevitable.

Finally, the consumption of products from Bt insect resistant plants raised some controversy regarding the possible long term effects of Bt on health. Although Betz et al. (2000) state that it has been used for over 40 years without causing adverse effects, the difference with GM plants is that Bt is not degraded in the plant and as a result both animals and humans may be exposed to this toxin (Aronson and Shai 2001).

Genotoxicity

Safety assessment for GM sweet pepper and tomato conferring resistance to cucumber mosaic virus showed no genotoxicity in animals (Chen et al., 2003). The use of lyophilized instead of raw GM food in this study may alter the toxicity results since there may be structural differences.

Pusztai's discipline of using animals with an acceptable starting weight range should be adopted in order to evaluate the toxic effects (Alliance for Biointegrity website 1998). The results of most studies with GM foods indicate that they may cause hepatic, pancreatic, and renal effects and may alter the hematological, biochemical, and immunologic parameters the significance of which remains to be solved with chronic toxicity studies.

Not only plants but animals as well have been genetically altered. The problems that may arise from the consumption of such products are also discussed.

EFFECTS OF INJECTED RECOMBINANT BOVINE GROWTH HORMONE (RBGH) IN ANIMALS

The use of rbGH in dairy cattle in order to increase milk yield has caused large controversy. Problems occurring such as an increase in mastitis may pose a risk to human health since the increased antibiotic use leads to antibiotic residues in milk (Epstein, 1996). Adverse effects in cows have been observed including lameness, mastitis, subclinical ketosis, an increase in embryonic loss and abortion, a decrease in final pregnancy rates, as well as a decrease in birth rate (Dohoo et al., 2003). It should be noted that lameness has also been reported in studies with transgenic pigs genetically engineered to carry human and bovine growth hormone genes (Pursel et al., 1989).

POSSIBLE RISKS FOR HUMAN HEALTH FROM THE USE OF MILK FROM COWS TREATED WITH RBGH

The consumption of milk from cows injected rbGH leads to an increase in IGF-I in humans, since IGF-1 survives digestion (Xian et al., 1995). The oral free IGF-1 feeding studies in rats sponsored by Monsanto and Elanco looked at by the Joint Expert Committee on Food Additives (JECFA) in 1992 had ambiguous results since neither used IGF-1 associated with its binding proteins, which are resistant to acidic conditions and may enable IGF-1 to survive digestion in the stomach. Moreover, IGF-1 is protected from digestion by the major milk protein casein (Hansen et al., 1997) and the milks buffering effect (Xian et al. 1995). Moreover, Monsanto's 90-day rat study which had previously shown that rbGH "is not orally active in rats" was re-examined and it was found that rbGH elicited a primary antigenic response meaning that rbGH was absorbed intact from the gut (Eppard et al., 1997). The full significance of human exposure to rbGH and IGF-1 is unknown, particularly in the neonate, the subpopulation at greatest risk (Morris, 1999). According to Chan (1998), at least some of the absorbed IGF-I can effectively stimulate the proliferation of cancer cells. The increased levels of IGF-I in humans predict increased rates in colon, breast, and prostate cancer, since they stimulate the indolent slowly growing tumor cells that appear in an aging individual resulting in clinical cancer necessarily old. On the other hand, FDA states that this potential does not exist since any increase of IGF-I in milk is much lower than the physiological amount produced in the organism. These concerns about the consumption of milk from cows injected rbGH may be carried also to other animals such as pigs expressing human GH, pigs injected recombinant porcine somatotropin (rpST), and GH transgenic salmon.

PIGS EXPRESSING HUMAN GROWTH HORMONE AND PIGS INJECTED RPST

Transgenic pigs expressing human GH showed dramatic effects in growth rates, feed conversion, and body composition, but exhibited serious side effects that were attributable to the high level of GH expression (Pursel et al., 1989). Repeated injections of rpST can also produce altered lipid composition similar to that of the GH transgenic pigs (Solomon et al., 1997).

GH TRANSGENIC FISH

Although the potential effect of feeding GM feed to poultry and cattle has been studied quite extensively (Einspanier et al., 2001; Hohlweg and Doerfler, 2001), there are only two available publications (Padgette et al., 1995; Hammond et al., 1996) in the case of fish feed. In both publications the effect of using GM ingredients in catfish feed, in terms of final fish weight and other physiognomic parameters, was investigated. Their conclusions were similar since the feeding values of GM soybeans and conventional soybeans were not found to be different. A more recent publication (Sanden et al., 2004) was focused both on: i) the fate of selected GM soy DNA fragments from feed to fish and on their survival through the fish gastrointestinal (GI) tract and ii) whether the DNA could be traced in a variety of fish tissues. Fish were fed in three experimental diets for six weeks, which were formulated from defined components and represented either GM or non-GM materials (17.2% of the fish meal was replaced with either GM or non-GM soy). A control diet composed of fish meal as the only protein source was used for comparison purposes. The transgenic sequences (120 and 195 bp) and the lectin gene (180 bp) could be detected in the GM soy feed. In the fish GI tract, however, only the smaller DNA fragment (120 bp) could be amplified from the content of the stomach, pyloric region, mid-intestine, and distal intestine. No transgenic or conventional soy DNA fragments could be detected in liver, muscle, or brain tissues dissected from sacrificed fish. The sensitivity limit of the method was evaluated to be 20 copies. Their data indicated that though GM soy transgenic sequences may survive passage through the GI tract, they could not be traced in fish tissues (Exadactylos and Arvanitoyannis, 2006).

However, when the fish growth hormone (GM) gene is introduced in salmon may GH circulation may elevate by 40-fold, leading to enlarged skulls and impair feeding and respiration (Dunham and Devlin, 1999). Experiments should be conducted in animals being fed GH transgenic salmon and other fish in order to examine whether the consumption of GH transgenic fish expressing high levels of GH will increase the levels of IGF-I and lead to the same health risks as rbGH milk. It should be emphasized that as in milk there is a possibility that the presence of other proteins in the fish tissue may protect IGF-1 from digestion, which remains to be demonstrated in animal studies.

Table 2 Comparison of values relevant to GE crops and foods among EU, Japan, Canada, and the USA (Arvanitoyannis, 2006)

Values	Importance of food safety	Environmental consciousness	Approach to science and technology	Attitude towards risk technology	Attitude towards food supply and trade
EU	Highly important but occurrence of diseases and contamination undermined the public trust	Very strong	Cautious	Medium	Strong but heavily opposed by environmental awareness
Japan	Highly important and public supports regulatory agencies' actions	Very strong	Innovative	Medium	Strong and linked with environmental awareness
Canada	Highly important and public encourages regulatory agencies' actions	Strong	Positive	Strong	Strong but mitigated by environmental awareness
USA	Highly important and public favours regulatory agencies' actions	Moderate	Enthusiastic	Very strong	Strong

GM PIG

The experiment of Saeki et al. (2004) with pigs containing spinach desaturase gene which converts saturated fat into the unsaturated fat linoleic acid resulted in a high degree of mortality in founders and the F_1 generation. Increased mortality might have been due to a random integration process where the transgene can insert in and damage any active gene locus (insertional mutagenesis) or to the significant alteration in the embryonic lipid profile caused by the transgene. The porcine embryo is unique in its high intracellular lipid content, which is associated with its sensitivity against freezing or in vitro production (Niemann and Rath, 2001). We strongly believe that the same toxicity could occur if the pregnant pigs were fed only the new source of g-linolenic acid obtained from transgenic canola or of any future modified crop, since it alters the percentage of 18:2n–6 in liver (Palombo et al., 2000). We should be aware that any change in the lipid profile of liver can also result in changes in metabolism with unexpected consequences.

ETHICS

The lasting sceptical and/or ambivalent attitude of Europeans towards agro-food biotechnology and the continued controversies about the commercialization of transgenic agro-food products are illustrative of an ongoing legitimacy crisis. One could even interpret the stigma on agro-food biotechnology and its products as testifying to a "robust" societal disapproval: it signals a lack of trust in scientific institutions and expert systems, and voices a social response against the reduction of the complexity of the GMO issue to a solely scientific risk-based problem. Hence, a move from a merely scientific evaluation towards a socially more robust one—that addresses precaution and socio-ethical issues in a more "sensible" way, whilst making "sense" of the different stances taken in the GMO debate—is still sought after. It will be interesting to see whether new controversies show (triggered, for example, by GMO contaminations or traces of unapproved transgenic events in nontransgenic produces), how these will be communicated and developed in the societal climate, and how they will be interpreted and tackled by, and/or lead to new adjustments in the now running legal system (Devos et al., 2007). The comparison of values relevant to GE crops and foods among EU, Japan, Canada, and the USA is given in Table 2.

CONCLUSIONS

From the review of the toxicity studies concerning GM foods one might see that although toxicity can be assessed, the duration of exposure is too short in order to fully evaluate any potential disruptions in biochemical parameters and to evidence possible signs of pathology within the limited subchronic exposure of animals. Moreover, a larger number of animals should be used in the toxicity tests. The toxicity tests should comply with the guidelines for toxicity testing of drugs. It should be emphasized that since these GM foods are going to be consumed by every human being they should be tested even more thoroughly than drugs and more experiments are required in order to study the possible toxicity and make any conclusions. Tests to determine how a GM food affects mutagenesis and carcinogenesis should be conducted as well. Finally, postmarketing surveillance should be part of the overall safety strategy for allergies, especially of high-risk groups such as infants and individuals in "atopic" families. Evaluation of protein allergenicity in man should also include studies in individuals not only with a history of allergy but with immunodeficiency as well. The use of recombinant GH in animals, such as cows or the expression of GH in animals such as salmon should be re-examined since it may promote cancer. The results of most of the rather few studies conducted with GM foods indicate that they may cause hepatic, pancreatic, renal, and reproductive effects and may alter hematological, biochemical, and immunologic parameters the significance of which remains unknown. The above results indicate that many GM food have some common toxic effects. Therefore, further studies should be conducted in order to elucidate the mechanism dominating this action. Small amounts of ingested DNA may not be broken down under digestive processes and there is a possibility that this DNA may either enter the bloodstream or be excreted, especially in individuals with abnormal digestion as a result of chronic gastrointestinal disease or with immunodeficiency.

Although intensive scientific effort is currently in progress to thoroughly understand and forecast possible consequences on humans, animals, and the environment, it is anticipated that

many years of careful, independent research with animals and clinical trials will be needed in order to accomplish this assessment.

ABBREVIATIONS

Bt	Bacillus thuringiensis
CaMV	Cauliflower Mosaic Virus
FAO	Food and Agriculture Organization of the United Nations
GFP	Green fluorescent protein
GM	Genetically modified
GNA	Galanthus nivalis
rGH	Recombinant growth hormone
WHO	World Health Organization

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ENSSER Statement, 21 October 2013
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No scientific consensus on GMO safety

As scientists, physicians, academics, and experts from disciplines relevant to the scientific, legal, social and safety assessment aspects of genetically modified organisms (GMOs),[1] we strongly reject claims by GM seed developers and some scientists, commentators, and journalists that there is a "scientific consensus" on GMO safety[2] [3] [4] and that the debate on this topic is "over".[5]

We feel compelled to issue this statement because the claimed consensus on GMO safety does not exist. The claim that it does exist is misleading and misrepresents the currently available scientific evidence and the broad diversity of opinion among scientists on this issue. Moreover, the claim encourages a climate of complacency that could lead to a lack of regulatory and scientific rigour and appropriate caution, potentially endangering the health of humans, animals, and the environment.

Science and society do not proceed on the basis of a constructed consensus, as current knowledge is always open to well-founded challenge and disagreement. We endorse the need for further independent scientific inquiry and informed public discussion on GM product safety and urge GM proponents to do the same.

Some of our objections to the claim of scientific consensus are listed below.

1. There is no consensus on GM food safety

[1] In the US, the term "genetically engineered" is often used in place of "genetically modified". We have used "genetically modified" because this is the terminology consistently used by many authorities internationally, including the Food and Agriculture Organization of the United Nations; the World Health Organization; Codex Alimentarius; European and Indian legislation; peer-reviewed studies by industry and independent scientists; and the international media. It is also consistent with the Cartagena Protocol's term "living modified organism".
[2] Frewin, G. (2013). The new "is GM food safe?" meme. Axis Mundi, 18 July. http://www.axismundionline.com/blog/the-new-is-gm-food-safe-meme/; Wikipedia (2013). Genetically modified food controversies. http://en.wikipedia.org/wiki/Genetically_modified_food_controversies
[3] Mark Lynas (2013). GMO pigs study – more junk science. Marklynas.org, 12 June. http://www.marklynas.org/2013/06/gmo-pigs-study-more-junk-science/
[4] Keith Kloor (2013). Greens on the run in debate over genetically modified food. Bloomberg, 7 January. http://www.bloomberg.com/news/2013-01-07/green-activist-reverses-stance-on-genetically-modified-food.html
[5] White, M. (2013). The scientific debate about GM foods is over: They're safe. Pacific Standard magazine, 24 Sept. http://www.psmag.com/health/scientific-debate-gm-foods-theyre-safe-66711/

Regarding the safety of GM crops and foods for human and animal health, a comprehensive review of animal feeding studies of GM crops found "An equilibrium in the number [of] research groups suggesting, on the basis of their studies, that a number of varieties of GM products (mainly maize and soybeans) are as safe and nutritious as the respective conventional non-GM plant, and those raising still serious concerns". The review also found that most studies concluding that GM foods were as safe and nutritious as those obtained by conventional breeding were "performed by biotechnology companies or associates, which are also responsible [for] commercializing these GM plants".[6]

A separate review of animal feeding studies that is often cited as showing that GM foods are safe included studies that found significant differences in the GM-fed animals. While the review authors dismissed these findings as not biologically significant,[7] the interpretation of these differences is the subject of continuing scientific debate[8] [9] [10] [11] and no consensus exists on the topic.

Rigorous studies investigating the safety of GM crops and foods would normally involve animal feeding studies in which one group of animals is fed GM food and another group is fed an equivalent non-GM diet. Independent studies of this type are rare, but when such studies have been performed, some have revealed toxic effects or signs of toxicity in the GM-fed animals.[12] [13] [14] [15] [16] [17] The concerns raised by these studies have not been followed up by targeted research that could confirm or refute the initial findings.

The lack of scientific consensus on the safety of GM foods and crops is underlined by the recent research calls of the European Union and the French

[6] Domingo, J. L. and J. G. Bordonaba (2011). A literature review on the safety assessment of genetically modified plants. Environ Int 37: 734–742.
[7] Snell, C., et al. (2012). Assessment of the health impact of GM plant diets in long-term and multigenerational animal feeding trials: A literature review. Food and Chemical Toxicology 50(3–4): 1134-1148.
[8] Séralini, G. E., et al. (2011). Genetically modified crops safety assessments: Present limits and possible improvements. Environmental Sciences Europe 23(10).
[9] Dona, A. and I. S. Arvanitoyannis (2009). Health risks of genetically modified foods. Crit Rev Food Sci Nutr 49(2): 164–175.
[10] Domingo, J. L. and J. G. Bordonaba (2011). Ibid.
[11] Diels, J., et al. (2011). Association of financial or professional conflict of interest to research outcomes on health risks or nutritional assessment studies of genetically modified products. Food Policy 36: 197–203.
[12] Domingo, J. L. and J. G. Bordonaba (2011). Ibid..
[13] Diels, J., et al. (2011). Ibid.
[14] Dona, A. and I. S. Arvanitoyannis (2009). Ibid.
[15] Séralini, G. E., et al. (2012). Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Food and Chemical Toxicology 50(11): 4221-4231.
[16] Séralini, G. E., et al. (2013). Answers to critics: Why there is a long term toxicity due to NK603 Roundup-tolerant genetically modified maize and to a Roundup herbicide. Food and Chemical Toxicology 53: 461-468.
[17] Carman, J. A., et al. (2013). A long-term toxicology study on pigs fed a combined genetically modified (GM) soy and GM maize diet. Journal of Organic Systems 8(1): 38–54.

government to investigate the long-term health impacts of GM food consumption in the light of uncertainties raised by animal feeding studies.[18] [19] These official calls imply recognition of the inadequacy of the relevant existing scientific research protocols. They call into question the claim that existing research can be deemed conclusive and the scientific debate on biosafety closed.

2. There are no epidemiological studies investigating potential effects of GM food consumption on human health

It is often claimed that "trillions of GM meals" have been eaten in the US with no ill effects. However, no epidemiological studies in human populations have been carried out to establish whether there are any health effects associated with GM food consumption. As GM foods are not labelled in North America, a major producer and consumer of GM crops, it is scientifically impossible to trace, let alone study, patterns of consumption and their impacts. Therefore, claims that GM foods are safe for human health based on the experience of North American populations have no scientific basis.

3. Claims that scientific and governmental bodies endorse GMO safety are exaggerated or inaccurate

Claims that there is a consensus among scientific and governmental bodies that GM foods are safe, or that they are no more risky than non-GM foods,[20] [21] are false.

For instance, an expert panel of the Royal Society of Canada issued a report that was highly critical of the regulatory system for GM foods and crops in that country. The report declared that it is "scientifically unjustifiable" to presume that GM foods are safe without rigorous scientific testing and that the "default prediction" for every GM food should be that the introduction of a new gene will cause "unanticipated changes" in the expression of other genes, the pattern of proteins produced, and/or metabolic activities. Possible outcomes of these changes identified in the report included the presence of new or unexpected allergens.[22]

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[18] EU Food Policy (2012). Commission and EFSA agree need for two-year GMO feeding studies. 17 December.

[19] French Ministry of Ecology, Sustainable Development and Energy (2013). Programme National de Recherche: Risques environnementaux et sanitaires liés aux OGM (Risk'OGM). 12 July. http://www.developpement-durable.gouv.fr/IMG/pdf/APR__Risk_OGM_rel_pbch_pbj_rs2.pdf

[20] Wikipedia (2013). Genetically modified food controversies. http://en.wikipedia.org/wiki/Genetically_modified_food_controversies

[21] G. Masip (2013). Opinion: Don't fear GM crops, Europe! The Scientist, May 28. http://www.the-scientist.com/?articles.view/articleNo/35578/title/Opinion--Don-t-Fear-GM-Crops--Europe-/

[22] Royal Society of Canada (2001). Elements of precaution: Recommendations for the regulation of Food Biotechnology in Canada; An Expert Panel Report on the Future of Food Biotechnology. January. http://www.rsc.ca//files/publications/expert_panels/foodbiotechnology/GMreportEN.pdf

A report by the British Medical Association concluded that with regard to the long-term effects of GM foods on human health and the environment, "many unanswered questions remain" and that "safety concerns cannot, as yet, be dismissed completely on the basis of information currently available". The report called for more research, especially on potential impacts on human health and the environment.[23]

Moreover, the positions taken by other organizations have frequently been highly qualified, acknowledging data gaps and potential risks, as well as potential benefits, of GM technology. For example, a statement by the American Medical Association's Council on Science and Public Health acknowledged "a small potential for adverse events … due mainly to horizontal gene transfer, allergenicity, and toxicity" and recommended that the current voluntary notification procedure practised in the US prior to market release of GM crops be made mandatory.[24] It should be noted that even a "small potential for adverse events" may turn out to be significant, given the widespread exposure of human and animal populations to GM crops.

A statement by the board of directors of the American Association for the Advancement of Science (AAAS) affirming the safety of GM crops and opposing labelling[25] cannot be assumed to represent the view of AAAS members as a whole and was challenged in an open letter by a group of 21 scientists, including many long-standing members of the AAAS.[26] This episode underlined the lack of consensus among scientists about GMO safety.

4. EU research project does not provide reliable evidence of GM food safety

An EU research project[27] has been cited internationally as providing evidence for GM crop and food safety. However, the report based on this project, "A Decade of EU-Funded GMO Research", presents no data that could provide such evidence, from long-term feeding studies in animals.

Indeed, the project was not designed to test the safety of any single GM food, but to focus on "the development of safety assessment approaches".[28] Only five published animal feeding studies are referenced in the SAFOTEST section of the

[23] British Medical Association Board of Science and Education (2004). Genetically modified food and health: A second interim statement. March. http://bit.ly/19QAHSI
[24] American Medical Association House of Delegates (2012). Labeling of bioengineered foods. Council on Science and Public Health Report 2. http://www.ama-assn.org/resources/doc/csaph/a12-csaph2-bioengineeredfoods.pdf
[25] AAAS (2012). Statement by the AAAS Board of Directors on labeling of genetically modified foods. 20 October. http://www.aaas.org/news/releases/2012/media/AAAS_GM_statement.pdf
[26] Hunt, P., et al. (2012). Yes: Food labels would let consumers make informed choices. Environmental Health News. http://www.environmentalhealthnews.org/ehs/news/2012/yes-labels-on-gm-foods
[27] European Commission (2010). A decade of EU-funded GMO research (2001–2010).
[28] European Commission (2010): 128.

report, which is dedicated to GM food safety.[29] None of these studies tested a commercialised GM food; none tested the GM food for long-term effects beyond the subchronic period of 90 days; all found differences in the GM-fed animals, which in some cases were statistically significant; and none concluded on the safety of the GM food tested, let alone on the safety of GM foods in general. Therefore the EU research project provides no evidence for sweeping claims about the safety of any single GM food or of GM crops in general.

5. List of several hundred studies does not show GM food safety

A frequently cited claim published on an Internet website that several hundred studies "document the general safety and nutritional wholesomeness of GM foods and feeds"[30] is misleading. Examination of the studies listed reveals that many do not provide evidence of GM food safety and, in fact, some provide evidence of a lack of safety. For example:

- Many of the studies are not toxicological animal feeding studies of the type that can provide useful information about health effects of GM food consumption. The list includes animal production studies that examine parameters of interest to the food and agriculture industry, such as milk yield and weight gain;[31] [32] studies on environmental effects of GM crops; and analytical studies of the composition or genetic makeup of the crop.
- Among the animal feeding studies and reviews of such studies in the list, a substantial number found toxic effects and signs of toxicity in GM-fed animals compared with controls.[33] [34] [35] [36] [37] [38] Concerns raised by these studies have not been satisfactorily addressed and the claim that the body

[29] European Commission (2010): 157.
[30] Tribe, D. (undated). 600+ published safety assessments. GMOPundit blog. http://gmopundit.blogspot.co.uk/p/450-published-safety-assessments.html
[31] Brouk, M., et al. (2008). Performance of lactating dairy cows fed corn as whole plant silage and grain produced from a genetically modified event DAS-59122-7 or a nontransgenic, near isoline control. J Anim. Sci, (Sectional Meeting Abstracts) 86(e-Suppl. 3):89 Abstract 276.
[32] Calsamiglia, S., et al. (2007). Effects of corn silage derived from a genetically modified variety containing two transgenes on feed intake, milk production, and composition, and the absence of detectable transgenic deoxyribonucleic acid in milk in Holstein dairy cows. J Dairy Sci 90: 4718-4723.
[33] de Vendômois, J.S., et al. (2010). A comparison of the effects of three GM corn varieties on mammalian health. Int J Biol Sci. ;5(7):706-26.
[34] Ewen, S.W.B. and A. Pusztai (1999). Effect of diets containing genetically modified potatoes expressing Galanthus nivalis lectin on rat small intestine. Lancet 354:1353-1354.
[35] Fares, N.H., and A. K. El-Sayed (1998). Fine structural changes in the ileum of mice fed on delta-endotoxin-treated potatoes and transgenic potatoes. Nat Toxins. 6:219-33.
[36] Kilic, A. and M. T. Akay (2008). A three generation study with genetically modified Bt corn in rats: Biochemical and histopathological investigation. Food Chem Toxicol 46(3): 1164–1170.
[37] Malatesta, M., et al. (2002). Ultrastructural morphometrical and immunocytochemical analyses of hepatocyte nuclei from mice fed on genetically modified soybean. Cell Structure and Function 27:173-180.
[38] Malatesta, M., et al. (2003). Fine structural analyses of pancreatic acinar cell nuclei from mice fed on genetically modified soybean. European Journal of Histochemistry 47:385-388

of research shows a consensus over the safety of GM crops and foods is false and irresponsible.

- Many of the studies were conducted over short periods compared with the animal's total lifespan and cannot detect long-term health effects.[39] [40]

We conclude that these studies, taken as a whole, are misrepresented on the Internet website as they do not "document the general safety and nutritional wholesomeness of GM foods and feeds". Rather, some of the studies give serious cause for concern and should be followed up by more detailed investigations over an extended period of time.

6. There is no consensus on the environmental risks of GM crops

Environmental risks posed by GM crops include the effects of Bt insecticidal crops on non-target organisms and effects of the herbicides used in tandem with herbicide-tolerant GM crops.

As with GM food safety, no scientific consensus exists regarding the environmental risks of GM crops. A review of environmental risk assessment approaches for GM crops identified shortcomings in the procedures used and found "no consensus" globally on the methodologies that should be applied, let alone on standardized testing procedures.[41]

Some reviews of the published data on Bt crops have found that they can have adverse effects on non-target and beneficial organisms[42] [43] [44] [45] – effects that are widely neglected in regulatory assessments and by some scientific commentators. Resistance to Bt toxins has emerged in target pests,[46] and problems with secondary (non-target) pests have been noted, for example, in Bt

[39] Hammond, B., et al. (2004). Results of a 13 week safety assurance study with rats fed grain from glyphosate tolerant corn. Food Chem Toxicol 42(6): 1003-1014.
[40] Hammond, B. G., et al. (2006). Results of a 90-day safety assurance study with rats fed grain from corn borer-protected corn. Food Chem Toxicol 44(7): 1092-1099.
[41] Hilbeck, A., et al. (2011). Environmental risk assessment of genetically modified plants - concepts and controversies. Environmental Sciences Europe 23(13).
[42] Hilbeck, A. and J. E. U. Schmidt (2006). Another view on Bt proteins – How specific are they and what else might they do? Biopesti Int 2(1): 1–50.
[43] Székács, A. and B. Darvas (2012). Comparative aspects of Cry toxin usage in insect control. Advanced Technologies for Managing Insect Pests. I. Ishaaya, S. R. Palli and A. R. Horowitz. Dordrecht, Netherlands, Springer: 195–230.
[44] Marvier, M., et al. (2007). A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. Science 316(5830): 1475-1477.
[45] Lang, A. and E. Vojtech (2006). The effects of pollen consumption of transgenic Bt maize on the common swallowtail, Papilio machaon L. (Lepidoptera, Papilionidae). Basic and Applied Ecology 7: 296–306.
[46] Gassmann, A. J., et al. (2011). Field-evolved resistance to Bt maize by Western corn rootworm. PLoS ONE 6(7): e22629.

cotton in China.[47] [48]

Herbicide-tolerant GM crops have proved equally controversial. Some reviews and individual studies have associated them with increased herbicide use,[49] [50] the rapid spread of herbicide-resistant weeds,[51] and adverse health effects in human and animal populations exposed to Roundup, the herbicide used on the majority of GM crops.[52] [53] [54]

As with GM food safety, disagreement among scientists on the environmental risks of GM crops may be correlated with funding sources. A peer-reviewed survey of the views of 62 life scientists on the environmental risks of GM crops found that funding and disciplinary training had a significant effect on attitudes. Scientists with industry funding and/or those trained in molecular biology were very likely to have a positive attitude to GM crops and to hold that they do not represent any unique risks, while publicly-funded scientists working independently of GM crop developer companies and/or those trained in ecology were more likely to hold a "moderately negative" attitude to GM crop safety and to emphasize the uncertainty and ignorance involved. The review authors concluded, "The strong effects of training and funding might justify certain institutional changes concerning how we organize science and how we make public decisions when new technologies are to be evaluated."[55]

7. International agreements show widespread recognition of risks posed by GM foods and crops

The Cartagena Protocol on Biosafety was negotiated over many years and implemented in 2003. The Cartagena Protocol is an international agreement ratified by 166 governments worldwide that seeks to protect biological diversity from the risks posed by GM technology. It embodies the Precautionary Principle

[47] Zhao, J. H., et al. (2010). Benefits of Bt cotton counterbalanced by secondary pests? Perceptions of ecological change in China. Environ Monit Assess 173(1-4): 985-994.
[48] Lu, Y., et al. (2010). Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. Science 328(5982): 1151-1154.
[49] Benbrook, C. (2012). Impacts of genetically engineered crops on pesticide use in the US – The first sixteen years. Environmental Sciences Europe 24(24).
[50] Heinemann, J. A., et al. (2013). Sustainability and innovation in staple crop production in the US Midwest. International Journal of Agricultural Sustainability: 1–18.
[51] Powles, S. B. (2008). Evolved glyphosate-resistant weeds around the world: Lessons to be learnt. Pest Manag Sci 64: 360–365.
[52] Székács, A. and B. Darvas (2012). Forty years with glyphosate. Herbicides - Properties, Synthesis and Control of Weeds. M. N. Hasaneen, InTech.
[53] Benedetti, D., et al. (2013). Genetic damage in soybean workers exposed to pesticides: evaluation with the comet and buccal micronucleus cytome assays. Mutat Res 752(1-2): 28-33.
[54] Lopez, S. L., et al. (2012). Pesticides used in South American GMO-based agriculture: A review of their effects on humans and animal models. Advances in Molecular Toxicology. J. C. Fishbein and J. M. Heilman. New York, Elsevier. 6: 41–75.
[55] Kvakkestad, V., et al. (2007). Scientists' perspectives on the deliberate release of GM crops. Environmental Values 16(1): 79–104.

in that it allows signatory states to take precautionary measures to protect themselves against threats of damage from GM crops and foods, even in case of a lack of scientific certainty.[56]

Another international body, the UN's Codex Alimentarius, worked with scientific experts for seven years to develop international guidelines for the assessment of GM foods and crops, because of concerns about the risks they pose. These guidelines were adopted by the Codex Alimentarius Commission, of which over 160 nations are members, including major GM crop producers such as the United States.[57]

The Cartagena Protocol and Codex share a precautionary approach to GM crops and foods, in that they agree that genetic engineering differs from conventional breeding and that safety assessments should be required before GM organisms are used in food or released into the environment.

These agreements would never have been negotiated, and the implementation processes elaborating how such safety assessments should be conducted would not currently be happening, without widespread international recognition of the risks posed by GM crops and foods and the unresolved state of existing scientific understanding.

Concerns about risks are well-founded, as has been demonstrated by studies on some GM crops and foods that have shown adverse effects on animal health and non-target organisms, indicated above. Many of these studies have, in fact, fed into the negotiation and/or implementation processes of the Cartagena Protocol and Codex. We support the application of the Precautionary Principle with regard to the release and transboundary movement of GM crops and foods.

Conclusion

In the scope of this document, we can only highlight a few examples to illustrate that the totality of scientific research outcomes in the field of GM crop safety is nuanced, complex, often contradictory or inconclusive, confounded by researchers' choices, assumptions, and funding sources, and in general, has raised more questions than it has currently answered.

Whether to continue and expand the introduction of GM crops and foods into the human food and animal feed supply, and whether the identified risks are acceptable or not, are decisions that involve socioeconomic considerations beyond the scope of a narrow scientific debate and the currently unresolved

[56] Secretariat of the Convention on Biological Diversity (2000). Cartagena Protocol on Biosafety to the Convention on Biological Diversity. http://bch.cbd.int/protocol/text/
[57] Codex Alimentarius (2009). Foods derived from modern biotechnology. 2d ed. World Health Organization/Food and Agriculture Organization of the United Nations. ftp://ftp.fao.org/codex/Publications/Booklets/Biotech/Biotech_2009e.pdf

biosafety research agendas. These decisions must therefore involve the broader society. They should, however, be supported by strong scientific evidence on the long-term safety of GM crops and foods for human and animal health and the environment, obtained in a manner that is honest, ethical, rigorous, independent, transparent, and sufficiently diversified to compensate for bias.

Decisions on the future of our food and agriculture should not be based on misleading and misrepresentative claims that a “scientific consensus” exists on GMO safety.

The document was first signed by 92 persons.

The document is now open for further signatures and all agreeing with the content are invited to sign the statement at: www.ensser.org

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Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis

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We report a population based case–control study of exposure to pesticides as risk factor for non-Hodgkin lymphoma (NHL). Male and female subjects aged 18–74 years living in Sweden were included during December 1, 1999, to April 30, 2002. Controls were selected from the national population registry. Exposure to different agents was assessed by questionnaire. In total 910 (91%) cases and 1016 (92%) controls participated. Exposure to herbicides gave odds ratio (OR) 1.72, 95% confidence interval (CI) 1.18–2.51. Regarding phenoxyacetic acids highest risk was calculated for MCPA; OR 2.81, 95% CI 1.27–6.22, all these cases had a latency period >10 years. Exposure to glyphosate gave OR 2.02, 95% CI 1.10–3.71 and with >10 years latency period OR 2.26, 95% CI 1.16–4.40. Insecticides overall gave OR 1.28, 95% CI 0.96–1.72 and impregnating agents OR 1.57, 95% CI 1.07–2.30. Results are also presented for different entities of NHL. In conclusion our study confirmed an association between exposure to phenoxyacetic acids and NHL and the association with glyphosate was considerably strengthened.

Key words: phenoxyacetic acids; MCPA; glyphosate; insecticides; impreganting agents; non-Hodgkin lymphoma

Non-Hodgkin lymphoma (NHL) is a heterogeneous group of lymphoid malignancies, where new classification systems based on immunohistochemistry, cytogenetics and evolving knowledge in clinical presentation and course has lead to modern classification systems.[1] Today, it is therefore more adequate to discuss NHL as many different diseases, which share some features but also differ in several aspects.

Interest in the etiology of NHL has been strengthened by an observed substantial increase in the incidence of the disease from the 1960's to the 1980's as reported from most countries with reliable cancer registries. However, this increase has clearly leveled off in many countries since the early 1990's, *i.e.*, in Sweden, Denmark and the USA.[2] The established risk factors for development of NHL include different immunosuppressive states, *e.g.*, human immunodeficiency virus (HIV), autoimmune diseases as Sjögren's syndrome and systemic lupus erythematosus (SLE), immunodepressants used after organ transplantation and some inherited conditions, for review see *e.g.*, Ref. 3. However, these causes may only explain a minority of cases, with a possible exception for HIV-related increases among younger persons in certain areas.[4]

It has been shown that Epstein-Barr virus (EBV) plays an essential role in the pathogenesis of lymphomas after organ transplantation.[5] A relation between lymphoma and elevated EBV-titers has been reported in a cohort.[6] Normally, EBV-production is held back by active cellular and humoral immune mechanisms. In immunodeficiency states this balance is disrupted and EBV-infected B-cells begin to proliferate.[7]

During the last decades, research on the etiology of NHL has been directed towards other potential causes such as pesticides, which may explain the impressive increase in the incidence. Today, it is also reasonable to consider the leveling off in incidence as a probable consequence of a reduced carcinogenic influence related to NHL. Furthermore, our emerging knowledge concerning the spectrum of NHL subgroups makes it reasonable to investigate causative agents for these different types of disease.

In 1981, we published results from a case–control study from Sweden, indicating statistically significant increased odds ratios for NHL and Hodgkin lymphoma (HL) in persons who had been exposed to phenoxyacetic herbicides or impregnating chlorophenols.[8] Our study was initiated by a case report.[9] Some of these chemicals were contaminated by dioxins, of which 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has been recognised as a complete carcinogen by IARC.[10] Furthermore, these and several other related chemicals are immunotoxic.[11–15] Our results have been confirmed in some other studies, regarding phenoxyacetic herbicides from *e.g.*, Kansas[16] and Nebraska.[17]

Furthermore, in 1999 we reported a new case–control study performed to evaluate more recent exposure to pesticides and other chemicals, and we could thereby confirm our earlier findings regarding a relation with phenoxyacetic herbicides that was related to latency period.[18]

In that study, however, some newer compounds that are widely used today, such as the herbicide glyphosate, were still not very common. During the 1970's certain chemicals, *e.g.*, the phenoxy herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), chlorophenols, and the insecticide dichlorodiphenyltrichloroethane (DDT), were prohibited due to health concerns. Later also the phenoxy herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was banned in Sweden. Reporting of these agents is therefore nowadays much less likely. It is also probable that the risk pattern has been influenced by protective measures during the last decades.

To further evaluate the relation between exposure to pesticides and other chemicals, focusing also on newer types of compounds, we have performed a new case–control study in Sweden. In our study we have also evaluated exposures in relation to different histopathological subtypes according to the most recent classification.[1]

Material and methods

The study covered 4 out of 7 health service regions in Sweden, associated with the University Hospitals in Lund, Linköping, Örebro and Umeå, and was approved by the ethics committees. Data were collected during December 1, 1999, to April 30, 2002, which was the time period for diagnosis of the cases. Regarding recruitment of cases and controls collaboration was established with another research group, which at the same time performed a parallel study on NHL in Sweden and Denmark.

Cases

All consecutive patients aged 18–74 years with newly diagnosed NHL, identified through physicians treating lymphoma and through pathologists diagnosing the disease, were approached if their physician did not judge this as less appropriate by ethical rea-

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sons. This was done regardless of whether the person had accepted to participate in the parallel study with which we collaborated in the recruitment procedure. If they accepted to participate they were included as potential cases, and went through the data assessment procedure described below. No cases were excluded because of specific conditions potentially associated with NHL, but no cases with *e.g.*, HIV or postransplantation NHL occurred. All the diagnostic pathological specimens were scrutinised by 1 out of 5 Swedish expert lymphoma reference pathologists, if they had not been initially judged by one of these 5. About 70% of all included cases were reviewed, whereas the remaining had been previously classified by one of the reference pathologists. If there was a disagreement from the original report the sample was reviewed by a panel of these pathologists. Therefore, some potential cases could later be excluded if a NHL diagnosis was not verified, and in those occasions all collected exposure information was disregarded. The pathologists also subdivided all NHL cases according to the WHO classification,[1] to enable etiological analyses also for the different diagnostic NHL entities. Since all lymphoma treating clinics and all lymphoma pathologists in the involved regions were covered by the study, it may well be regarded as population based, although the possibility of some individuals not reported through the case ascertainment system used.

Controls

From the population registry covering whole Sweden, randomly chosen controls living in the same health service regions as the cases were recruited during several occasions within the study period. The controls were frequency-matched in 10 years age and sex groups to mirror the age and sex distribution of the included cases, and to increase efficacy in the adjusted analyses. If they accepted to participate, they were included as controls.

Assessment of exposure

All subjects who accepted to participate received a comprehensive questionnaire, which was sent out shortly after the subjects had been telephone interviewed by the other research group we had collaboration with as stated earlier. Their interview, however, did not focus on work environment or chemical exposure, but rather dealt with other life style factors and diseases. Our questionnaire included a total work history with in depth questions regarding exposure to pesticides, organic solvents and several other chemicals. For all pesticides not only numbers of years and numbers of days per year, but also approximate length of exposure per day were questioned. Since most work with pesticides was performed in an individualized manner, no job-exposure matrix was judged to be applicable. Furthermore, the questionnaire also included questions on *e.g.*, smoking habits, medications, leisure time activities and proximity from home to certain industrial installations, but data on these factors are not included in this article.

Specially trained interviewers scrutinized the answers and collected additional exposure information by phone if important data were lacking, incomplete or unclear. These interviewers were blinded with regard to case/control status. All exposures during the same calendar year as the diagnosis and the year before were disregarded in the cases. Correspondingly, the year of enrolment and the year before were disregarded for the controls. As in our previous lymphoma studies we used a minimum criterion of one full day exposure to be categorized as exposed.[8,18]

Statistical methods

Unconditional logistic regression analysis (Stata/SE 8.2 for Windows; StataCorp, College Station, TX) was used to calculate odds ratios (OR) and 95% confidence intervals (CI). Adjustment was made for age, sex and year of diagnosis (cases) or enrolment (controls). In the univariate analysis, different pesticides were analyzed separately and the unexposed category consisted of subjects that were unexposed to all included pesticides. When analyzing subgroups of NHL all controls were used in the separate analyses. In the dose-response calculations made for agents with at least 20 exposed subjects, median number of days of exposure among controls was used as cut-off. Latency period calculations and multivariate analyses included agents with statistically significant increased OR, or with an OR $>$ 1.50 and at least 10 exposed subjects.

TABLE I – NON-HODGKIN LYMPHOMA CASES DIVIDED ON HISTOPATHOLOGICAL SUBTYPES ACCORDING TO WHO CLASSIFICATION.

WHO diagnosis	Number of cases
B-cell lymphomas, total	819
Lymphocytic lymphoma/B-CLL (SLL/CLL)	195
Follicular, grade I–III (FL)	165
Diffuse large B-cell lymphoma (DLBCL)	239
Other specified B-cell lymphoma	131
Unspecified B-cell lymphoma	89
T-cell lymphomas	53
Unspecified non-Hodgkin lymphoma	38
Total	910

Results

In total, 1,163 cases were reported from the participating clinics. Of these, 46 could not participate because of medical conditions, 88 died before they could be interviewed. Since these were primarily excluded by the reporting physicians we had no information on *e.g.*, final WHO categories on these cases. Three NHL cases were not diagnosed during the study period, 1 lived outside the study area and 30 were excluded not being NHL (HL 20, acute lymphoblastic leukaemia 1, other malignancy 7 and unclear diagnosis 2). Of the finally included 995 cases with NHL, 910 (91%) accepted to participate and answered the questionnaire. Of these, 819 were B-cell, 53 T-cell and 38 unspecified lymphomas, Table I.

Among the 1,108 initially enrolled controls 92 did not respond to the mail questionnaire, resulting in 1,016 (92%) controls to be included in the analyses.

The medium and median age in cases was 60 and 62 years, and in controls it was 58 and 60 years, respectively. Of the cases, 534 were males and 376 females, and of the controls the corresponding numbers were 592 and 424.

This report presents exposure data regarding different types of pesticides.

Herbicides

Exposure to herbicides gave for all NHL OR 1.72 (95% CI 1.18–2.51), Table II. Exposure to phenoxyacetic acids yielded OR 2.04 (95% CI 1.24–3.36). This group was further subdivided in 3 categories; (*i*) 4-chloro-2-methyl phenoxyacetic acid (MCPA), which is still on the market and not known to be contaminated by dioxins; (*ii*) 2,4,5-T and/or 2,4-D which often were used together and were potentially contaminated with different dioxin isomers; (*iii*) other types. MCPA seemed to give the most pronounced increase in OR. Exposure to other herbicides, regardless if they also had been exposed to phenoxyacetic acids or not, also gave a statistically significant OR 1.82 (95% CI 1.08–3.06). In this category the dominating agent was glyphosate, which was reported by 29 cases and 18 controls, which produced OR 2.02 (95% CI 1.10–3.71). If both phenoxyacetic acids and glyphosate were excluded, exposure to other herbicides (37 different agents reported, but no one by more than 6 subjects at most) gave a nonsignificant OR of 1.22 (95 % CI 0.63–2.39).

Dose-response analyses regarding herbicides in total and glyphosate yielded an increased OR in the higher exposed group, Table II. For phenoxyacetic acids, however, no such association was demonstrated.

Regarding phenoxy herbicides and glyphosate an analysis was made taken the latency period for exposure into account. For the

latency period 1–10 years no exposed cases were found for MCPA and 2,4,5-T and/or 2,4-D. Regarding glyphosate OR 1.11 (95% CI 0.24–5.08) was obtained. Latency period >10 years yielded for MCPA OR 2.81 (95% CI 1.27–6.22), for 2,4,5-T and/or 2,4,-D OR 1.72 (95% CI 0.98–3.19), and for glyphosate OR 2.26 (95% CI 1.16–4.40).

When different NHL entities were analysed separately, the OR for the subtype small lymphocytic lymphoma/chronic lymphocytic leukaemia (SLL/CLL) was increased for both phenoxy herbicides and, especially, glyphosate, Table III. The entity diffuse large B-cell lymphoma (DLBCL) was significantly associated with exposure to phenoxyacetic acids, but not to other herbicides. On the other hand, the group follicular lymphoma was not clearly associated with phenoxyacetic acids, and only nonsignificantly with glyphosate. The category "other specified B-cell lymphoma" (*e.g.*, mantle cell lymphoma, marginal zone lymphoma) was significantly associated with exposure to phenoxyacetic acids, and an increased risk was also indicated for glyphosate. T-cell lymphomas seemed to be associated with all types of herbicides, but no statistically significant ORs were found due to relatively few exposed subjects. The least numerous categories ("unspecified NHL") yielded high and statistically significant ORs for phenoxy herbicides and glyphosate.

TABLE II – EXPOSURE TO VARIOUS HERBICIDES

Agents	Cases/controls	OR	CI
Herbicides, total	74/51	1.72	1.18–2.51
≤20 days	36/27	1.58	0.95–2.65
>20 days	38/24	1.87	1.10–3.18
Phenoxyacetic acids	47/26	2.04	1.24–3.36
≤45 days	32/13	2.83	1.47–5.47
>45 days	15/13	1.27	0.59–2.70
MCPA	21/9	2.81	1.27–6.22
≤32 days	15/5	3.76	1.35–10.5
>32 days	6/4	1.66	0.46–5.96
2,4,5-T and/or 2,4-D	33/21	1.61	0.87–2.97
≤29 days	21/11	2.08	0.99–4.38
>29 days	12/10	1.33	0.57–3.13
Other	7/7	1.21	0.42–3.48
Herbicides except phenoxyacetic acids	38/26	1.82	1.08–3.06
≤24 days	20/13	1.91	0.93–3.89
>24 days	18/13	1.73	0.84–3.60
Glyphosate	29/18	2.02	1.10–3.71
≤10 days	12/9	1.69	0.70–4.07
>10 days	17/9	2.36	1.04–5.37
Other herbicides	18/18	1.22	0.63–2.39
≤32 days	12/9	1.64	0.68–3.96
>32 days	6/9	0.80	0.28–2.29

Number of exposed cases/controls, odds ratios (OR) and 95% confidence intervals (CI). Agents with more than 20 exposed subjects were also divided in two groups based on median number of days among exposed controls. Adjustment was made for age, sex and year of diagnosis or enrolment.

Insecticides

In our study no overall increased OR was demonstrated for exposure to insecticides, OR 1.28 (95% CI 0.96–1.72), Table IV. The most reported insecticide DDT yielded OR 1.46 (95% CI 0.94–2.28). Increased risk was shown for mercurial seed dressing, OR 2.03 (95% CI 0.97–4.28).

In the dose-response analysis, OR 1.47 (95% CI 0.99–2.16) was found for the high category of insecticide exposure, Table IV. Similar trends were found for DDT and mercurial seed dressing.

Different NHL entities were analysed separately, Table V. Hereby, certain exposures seemed to be associated with subtypes of NHL. Thus, the group follicular lymphoma was associated with DDT, OR 2.14 (95% CI 1.05–4.40) and mercurial seed dressing, OR 3.61 (95% CI 1.20–10.9). Furthermore, exposure to DDT increased the risk also for T-cell lymphoma, OR 2.88 (95% CI 1.05–7.95).

Fungicides and rodenticides

Exposure to fungicides was not a risk factor in our study, neither in total, OR 1.11 (95% CI 0.56–2.23), Table IV, nor for different subtypes of NHL, Table VI. Furthermore, there were no single substances among 24 reported that significantly differed between cases and controls. Also for rodenticides no increased risk was found, Table IV.

Impregnating agents

Exposure to impregnating agents yielded a statistically significant OR 1.57 (95% CI 1.07–2.30), Table IV. In a dose-response calculation OR increased further in the high exposure group. Creosote showed a statistically significant OR for high exposure, OR 3.33 (95% CI 1.20–9.27).

Table VI presents results for different NHL entities. An increased risk for SLL/CLL was associated with exposure to impregnating agents in total, and most pronounced for creosote,

TABLE III – EXPOSURE TO VARIOUS HERBICIDES DIVIDED ACCORDING TO DIFFERENT LYMPHOMA ENTITIES

Lymphoma entities	Herbicides, total	Phenoxyacetic acids (ph)	MCPA	2,4,5-T and/or 2,4-D	Herbicides except ph	Glyphosate	Other
B-cell lymphomas, total (n = 819)	1.68 1.14–2.48	1.99 1.20–3.32	2.59 1.14–5.91	1.69 0.94–3.01	1.72 1.003–2.94	1.87 0.998–3.51	1.14 0.57–2.31
Lymphocytic lymphoma/B-CLL (n = 195) (SLL/CLL)	2.27 1.28–4.01	2.11 0.995–4.47	2.57 0.74–8.97	1.93 0.85–4.41	2.56 1.17–5.60	3.35 1.42–7.89	1.39 0.45–4.31
Follicular, grade I–III (n = 165) (FL)	1.78 0.88–3.59	1.26 0.42–3.75	–[1]	1.21 0.35–4.22	2.32 0.96–5.60	1.89 0.62–5.79	1.48 0.42–5.23
Diffuse large B-cell lymphoma (n = 239) (DLBCL)	1.44 0.81–2.59	2.16 1.08–4.33	3.94 1.48–10.5	1.65 0.71–3.82	1.20 0.51–2.83	1.22 0.44–3.35	1.00 0.33–3.03
Other specified B-cell lymphoma (n = 131)	1.62 0.82–3.19	2.60 1.20–5.64	3.20 0.95–10.7	2.21 0.90–5.44	1.38 0.51–3.73	1.63 0.53–4.96	1.15 0.33–4.03
Unspecified B-cell lymphoma (n = 89)	1.09 0.41–2.89	1.14 0.33–3.95	1.35 0.16–11.2	0.88 0.20–3.92	1.52 0.44–5.27	1.47 0.33–6.61	0.71 0.09–5.53
T-cell lymphomas (n = 53)	1.64 0.55–4.90	1.62 0.36–7.25	2.40 0.29–20.0	1.02 0.13–7.95	1.57 0.35–6.99	2.29 0.51–10.4	2.24 0.49–10.3
Unspecified non-Hodgkin lymphoma (n = 38)	2.86 1.001–8.18	3.75 1.16–12.1	9.31 2.11–41.2	3.21 0.85–12.1	5.29 1.60–17.5	5.63 1.44–22.0	1.88 0.23–15.4

Odds ratios (OR) and 95% confidence intervals (CI). Adjustment was made for age, sex and year of diagnosis or enrolment.
[1]No exposed cases

OR 2.91 (95% CI 1.01–8.33). Regarding follicular lymphomas and DLBCL, increased risks were also noted after creosote exposure, and for the latter subtype this was also the case for all impregnating agents together. T-cell lymphomas were also associated with impregnating agents, and it seemed to be specifically chlorophenols. In the group of patients whose lymphomas were not possible to classify histopathologically, increased risks were indicated for all types of impregnating agents.

TABLE IV – EXPOSURE TO VARIOUS OTHER PESTICIDES

Agents	Cases/controls	OR	CI
Insecticides, total	112/101	1.28	0.96–1.72
≤40 days	44/51	1.03	0.68–1.57
>40 days	65/50	1.47	0.99–2.16
DDT	50/37	1.46	0.94–2.28
≤37 days	20/19	1.17	0.62–2.22
>37 days	30/18	1.76	0.97–3.20
Mercurial seed dressing	21/11	2.03	0.97–4.28
≤12 days	7/6	1.27	0.42–3.83
>12 days	14/5	2.93	1.04–8.25
Pyretrine	15/10	1.74	0.78–3.91
≤25 days	8/5	1.86	0.60–5.75
>25 days	6/5	1.36	0.41–4.51
Permetrine	9/9	1.23	0.48–3.14
Other insecticides	28/26	1.25	0.72–2.16
≤33 days	9/14	0.79	0.34–1.85
>33 days	18/12	1.67	0.79–3.51
Fungicides	16/18	1.11	0.56–2.23
≤37 days	9/9	1.29	0.51–3.31
>37 days	7/9	0.94	0.35–2.57
Impregnating agents	70/51	1.57	1.07–2.30
≤45 days	27/25	1.23	0.71–2.16
>45 days	43/24	2.04	1.21–3.42
Chlorophenols	40/36	1.24	0.77–1.98
≤33 days	23/18	1.46	0.78–2.74
>33 days	17/17	1.08	0.54–2.15
Arsenic	7/5	1.63	0.51–5.20
Creosote	19/10	2.10	0.96–4.58
≤39 days	4/5	0.87	0.23–3.29
>39 days	15/5	3.33	1.20–9.27
Tar	8/5	1.84	0.59–5.69
Other impregnating agents	27/20	1.55	0.85–2.81
≤7 days	4/10	0.44	0.14–1.42
>7 days	22/10	2.55	1.19–5.47
Rodenticides	5/4	1.67	0.44–6.29

Number of exposed cases/controls, odds ratios (OR) and 95% confidence intervals (CI). Agents with more than 20 exposed subjects were also divided in two groups based on median number of days among exposed controls. In some subjects, number of days was not known (excluded in dose-response calculations). Adjustment was made for age, sex and year of diagnosis or enrolment.

Multivariate analysis

Since mixed exposure to several pesticides was more a rule than an exception, and all single agents were analyzed without adjusting for other exposure, a multivariate analysis was made to elucidate the relative importance of different pesticides. Criteria for agents to be included in this analysis are defined in Statistical Methods above. As seen in Table VII increased ORs were found but in general lower than in the univariate analysis.

Discussion

This was a population based case–control study on NHL, which is a strength of the investigation. Only living cases and controls were included, which was of advantage in comparison with interviewing next-of-kins. The study covered all new cases of NHL during a specified time. Pathologists in Sweden that were experts in lymphoma diagnosis confirmed all diagnoses. Thus, a main advantage compared with the earlier studies was the possibility to study the different NHL entities, classified according to the recently developed WHO classification system. The histopathological subgroups may well be regarded as separate in etiology and pathogenesis, as well as they are known to be different regarding course, prognosis and best treatment.

The frequency matching on age groups, gender and health service regions increased the efficacy of the study and ensured exposure conditions for the controls representative for the population in the included geographical areas. We achieved a high response rate among cases and controls, which is another advantage. A motivating introduction letter that was sent out with the questionnaire and with reminders if needed may explain this.

Exposures were assessed by questionnaires with information supplemented over the phone. Thereby use of different pesticides could be checked by information in *e.g.*, receipts and bookkeeping. However, no registries exist in Sweden on such individual use, which is a weakness in the assessment of exposure. Exposure to pesticides may be difficult to assess, and some misclassification regarding quantity of exposure has probably occurred, but such misclassification would most probably be nondependent of case/control status, and therefore only weaken any true risks. Use of protective equipment was not asked for which might have been a disadvantage of the study. However, such use would dilute the exposure and thus bias the result towards unity.

We have earlier published the results from 2 Swedish case–control studies on lymphomas, the first one on NHL and HL[8,19] and later on NHL.[18] These studies showed an increased risk for lymphomas as a result of exposure to herbicides belonging to the class phenoxyacetic acids. In the first study we also found correlation with chlorophenols and organic solvents. Several other studies,

TABLE V – EXPOSURE TO VARIOUS INSECTICIDES DIVIDED ACCORDING TO DIFFERENT LYMPHOMA ENTITIES

Lymphoma entities	Insecticides, total	DDT	Mercurial seed dressing	Pyretrine	Other
B-cell lymphomas, total (n = 819)	1.19	1.32	1.81	1.68	1.08
	0.88–1.61	0.83–2.10	0.84–3.93	0.73–3.86	0.60–1.94
Lymphocytic lymphoma/B-CLL (n = 195) (SLL/CLL)	1.46	1.39	0.75	2.40	1.57
	0.91–2.35	0.69–2.83	0.16–3.47	0.73–7.89	0.66–3.75
Follicular, grade I–III (n = 165) (FL)	1.37	2.14	3.61	2.60	0.28
	0.79–2.38	1.05–4.40	1.20–10.9	0.79–8.51	0.04–2.11
Diffuse large B-cell lymphoma (n = 239) (DLBCL)	1.23	1.24	2.20	1.25	1.31
	0.78–1.93	0.61–2.49	0.79-6.12	0.34–4.61	0.58–2.97
Other specified B-cell lymphoma (n = 131)	1.32	1.33	2.39	1.49	1.42
	0.77–2.27	0.57–3.10	0.73–7.81	0.32–6.94	0.53–3.80
Unspecified B-cell lymphoma (n = 89)	0.42	0.23	–[1]	–[1]	0.42
	0.15–1.18	0.03–1.75			0.06–3.18
T-cell lymphomas (n = 53)	1.61	2.88	2.08	2.20	1.59
	0.72–3.60	1.05–7.95	0.25–17.1	0.27–17.8	0.36–7.02
Unspecified non-Hodgkin lymphoma (n = 38)	1.91	2.39	5.43	3.14	4.70
	0.79–4.62	0.77–7.42	1.34–22.0	0.37–26.3	1.48–14.9

Odds ratios (OR) and 95% confidence intervals (CI). Adjustment was made for age, sex and year of diagnosis or enrolment.
[1]No exposed cases.

TABLE VI – EXPOSURE TO FUNGICIDES AND IMPREGNATING AGENTS DIVIDED ACCORDING TO DIFFERENT LYMPHOMA ENTITIES

Lymphoma entities	Fungicides	Impregnating agents, total	Chlorophenols	Creosote	Other
B-cell lymphomas, total (n = 819)	1.01	1.41	1.12	2.09	1.51
	0.48–2.09	0.95–2.11	0.69–1.84	0.94–4.64	0.82–2.78
Lymphocytic lymphoma/B-CLL (n = 195)	1.33	1.71	1.35	2.91	2.23
	0.43–4.12	0.94–3.11	0.64–2.85	1.01–8.33	0.97–5.13
Follicular, grade I–III (n = 165)	–[1]	1.49	0.91	2.56	1.80
		0.70–3.19	0.31–2.66	0.68–9.68	0.59–5.48
Diffuse large B-cell lymphoma (n = 239)	1.26	1.70	1.40	1.75	1.51
	0.45–3.47	0.97–2.96	0.70–2.78	0.54–5.74	0.62–3.67
Other specified B-cell lymphoma (n = 131)	1.56	1.24	0.95	2.58	1.09
	0.51–4.76	0.58–2.63	0.36–2.51	0.78–8.55	0.31–3.78
Unspecified B-cell lymphoma (n = 89)	–[1]	0.41	0.54	–[1]	0.54
		0.10–1.75	0.12–2.32		0.07–4.19
T-cell lymphomas (n = 53)	1.10	3.26	2.39	–[1]	2.07
	0.14–8.70	1.39–7.63	0.78–7.28		0.45–9.53
Unspecified non-Hodgkin lymphoma (n = 38)	3.73	2.52	2.02	4.94	1.40
	0.77–18.0	0.88–7.19	0.56–7.31	0.97–25.2	0.17–11.2

Odds ratios (OR) and 95% confidence intervals (CI). Adjustment was made for age, sex, and year of diagnosis or enrolment.
[1]No exposed cases.

TABLE VII – MULTIVARIATE ANALYSES INCLUDING AGENTS ACCORDING TO SPECIFIED CRITERIA, SEE TEXT

Agents	Univariate		Multivariate	
	OR	CI	OR	CI
MCPA	2.81	1.27–6.22	1.88	0.77–4.63
2,4,5-T and/or 2,4-D	1.61	0.87–2.97	1.24	0.68–2.26
Glyphosate	2.02	1.10–3.71	1.51	0.77–2.94
Mercurial seed dressing	2.03	0.97–4.28	1.58	0.74–3.40
Arsenic	1.63	0.51–5.20	1.17	0.34–4.02
Creosote	2.10	0.96–4.58	1.70	0.73–3.98
Tar	1.84	0.59–5.69	1.39	0.43–4.48

Odds ratios (OR) and 95% confidence intervals (CI). Adjustment was made for age, sex and year of diagnosis or enrolment.

but not all, from different research groups have supported our results, as reviewed,[20] and also confirmed later, *e.g.*, Ref. 21.

Furthermore, other groups have demonstrated associations between NHL and other classes of pesticides, especially different types of insecticides, *e.g.*, organophosphates,[22] carbamate,[23] lindane[24] and chlordane,[25] but also other groups of herbicides as atrazine.[26] Some case–control studies have found associations between several classes of pesticides, *e.g.*, Ref. 27 or merged groups of pesticides as in one recent study,[28] which demonstrate a significantly increased risk for NHL associated with exposure to "nonarsenic pesticides." These authors discuss the fact that several pesticides are chemically related and may exert their effects on humans through a similar mechanism of action, which may explain the wide range of pesticides that have been related to NHL over time in different countries and with different exposure conditions.

Several factors urged for a third Swedish study on the relation between pesticides, other chemicals and NHL, and the present study also used a somewhat changed methodology, which also may be of interest.

Thus, the use of phenoxyacetic herbicides, which earlier were dominating both as weed killers in agriculture and against hard wood in forestry, have substantially decreased during the last decades. 2,4,5-T, which was contaminated by TCDD, was prohibited in Sweden 1977, and 2,4-D was withdrawn from the market in 1990. MCPA, even if still used, has been largely substituted by other agents, among which glyphosate has been clearly dominating. This change of herbicide practice along with successively strengthened protection instructions has prompted our new study, reflecting also later years of exposure.

Furthermore, the changing trend of the incidence of NHL in many countries with reliable cancer registries, *e.g.*, Sweden, with a substantial and steady increase during the 1960's through 1980's but a leveling off or even slight decrease after that, makes it important to find etiological factors contributing to this shift in trend. Chlorinated compounds in the environment, which have been regulated during the 1970's and 1980's, may at least partly explain this trend, as discussed by us.[2] Phenoxyacetic herbicides with potential contaminating dioxins are examples of such substances. However, the prohibition of common environmental pollutants as polychlorinated biphenyls (PCB) and the following decline in the environment is probably more important to explain the leveling off of the incidence.[2]

In contrast to our 2 former case–control studies on NHL, this study included both genders and only consecutive living cases and living controls. In our earlier studies we have only studied male lymphoma cases, making the results of this study more representative for the whole population. To facilitate comparisons with our earlier results we also made additional analyses of herbicide exposure by gender. Only few women were exposed and separate analyses for both sexes still yielded an increased risk for NHL. Thus, in the total material herbicide exposure gave OR = 1.72, 95% CI 1.18–2.51 (n = 74 cases, 51 controls), whereas for men only OR = 1.71, 95% CI = 1.15–2.55 (n = 68 cases, 47 controls) and for women only OR = 1.82, 95% CI = 0.51–6.53 (n = 6 cases, 4 controls) were calculated.

In our study lymphocytic lymphoma/B-CLL was significantly associated with herbicides with highest OR for glyphosate but also creosote. Follicular lymphoma was significantly associated with DDT and mercurial seed dressing, diffuse large B-cell lymphoma with MCPA, and T-cell lymphoma with DDT and impregnating agents overall. Unspecified NHL was significantly associated with MCPA, glyphosate and mercurial seed dressing. It should be noted that several ORs were increased for herbicides; insecticides and impregnating agents but the calculations were hampered by low numbers of exposed cases and controls.

Our earlier results of exposure to phenoxyacetic herbicides as a risk factor for NHL were confirmed in our study. As in our previous lymphoma studies exposure to MCPA seemed to yield the highest OR among the different phenoxyacetic acids. This is of interest because MCPA is known not to be contaminated by dioxins, as 2,4-D and 2,4,5-T. At the same time MCPA is the only phenoxyacetic acid still in wider use in Sweden and many other countries.

Glyphosate is a broad-spectrum herbicide, which inhibits the formation of amino acids in plants.[29] The US Environmental Protection Agency[30] and the World Health Organization[31] have concluded that glyphosate is not mutagenic or carcinogenic. Since then, however, some experimental studies indicate genotoxic, hormonal and enzymatic effect in mammals, as reviewed.[32] Of particular interest is that glyphosate treatment of human lymphocytes *in vitro* resulted in increased sister chromatid exchanges,[33] chromosomal aberrations and oxidative stress.[34,35]

Glyphosate was associated with a statistically significant increased OR for lymphoma in our study, and the result was strengthened by a tendency to dose-response effect as shown in Table II. In our former study[18] very few subjects were exposed to glyphosate, but a nonsignificant OR of 2.3 was found. Furthermore, a meta-analysis combining that study with an investigation on hairy-cell leukaemia, a rare NHL variant, showed an OR for glyphosate of 3.04 (95% CI 1.08–8.52).[36] Recent findings from other groups also associate glyphosate with different B-cell malignancies such as lymphomas and myeloma.[32,37,38]

Glyphosate has succeeded MCPA as one of the most used herbicides in agriculture, and many individuals that used MCPA earlier are now also exposed to glyphosate. This probably explains why the multivariate analysis does not show any significant ORs for these compounds.

Exposure to insecticides was associated with a slightly increased OR, Table IV. In some other studies on the relation between pesticides and NHL, insecticides seem to be of some importance as causative agents.[27,37,38] Especially, different organophosphates were indicated as risk factors in those studies, with a Canadian study[37] showing statistical significant ORs for malathion and diazinon. In our study, only few subjects were exposed to different organophosphates, but we found a nonsignificant OR of 2.81 (95% CI 0.54–14.7) for malathion based on 5 exposed cases and 2 controls, not shown in Table.

The organochlorine DDT has shown suggestive but rarely significant association with NHL in some studies.[8,19,38–40] Our study showed a moderately but not significant increased OR for exposure to DDT.

Fungicides were not associated with the risk for NHL in our study, but few subjects were exposed to a wide range of different agents. In some earlier studies increased risks have also been noted for this group of pesticides.[16,18]

Exposure to impregnating agents produced a significant OR with a dose-response relation, Table IV. The highest risk was found for high exposure to creosote, which gave a significant OR. This finding was in contrast to our previous results on NHL,[18] but another Swedish study also found an association between creosote and NHL.[41] Chlorophenols have been the most common group of impregnating agents in Sweden, but were banned in 1977. In our first NHL study, reflecting exposures mainly during the time these substances were used, we found a strong association with NHL. As in the present study, however, no association was found in our second study on NHL.[18]

In conclusion, this study, which mirrors pesticide exposure during later years than in our previous studies, confirmed results of an association between exposure to phenoxyacetic herbicides and NHL. Furthermore, our earlier indication of an association between glyphosate and NHL has been considerably strengthened.

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Effect of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine

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See Commentaries pages 1314, 1315

Diets containing genetically modified (GM) potatoes expressing the lectin *Galanthus nivalis* agglutinin (GNA) had variable effects on different parts of the rat gastrointestinal tract. Some effects, such as the proliferation of the gastric mucosa, were mainly due to the expression of the GNA transgene. However, other parts of the construct or the genetic transformation (or both) could also have contributed to the overall biological effects of the GNA-GM potatoes, particularly on the small intestine and caecum.

Genetically modified (GM) plant products are becoming increasingly common in the human food-chain, yet in contrast to the general acceptance of the need for the biological testing of novel foods and feedstuffs, few studies have been carried out on the possible effects of GM products on the mammalian ut mucosa. GM potatoes expressing a snowdrop lectin (*Galanthus nivalis* agglutinin [GNA]) under the CaMV35s promoter have been developed to increase insect and nematode resistance.[1] GNA was selected for insertion into potatoes because the initial effect of this mannose-specific lectin on the rat small bowel has been shown to be minimal,[2] and because its binding to mannose present on the epithelial surface of rat jejunal villi is demonstrable only after feeding for 10 days. We compared the histological indices of the gut of rats fed potato diets containing GM potatoes, non-GM potatoes, or non-GM potatoes supplemented with GNA, to find out whether GNA gene insertion had affected the nutritional and physiological impact of potatoes on the mammalian gut.

ELISA analysis confirmed that the expression level of GNA in raw GM potatoes was 25·4 μg/g dry matter; the concentration was decreased to 4·9 μg/g after boiling for 1 h. Six rats were randomly allocated to each group, and were fed diets containing either raw or boiled GNA-GM potatoes, parent potatoes (Desiree), or parent-line potatoes supplemented with 25·4 μg/g GNA for 10 days. All potato diets were isocaloric and contained an average of 6% protein. Histological samples of stomach, jejunum, ileum, caecum, and colon were taken 10 days after the start of feeding. The samples, each 2 cm in length, were opened along the antimesenteric border. The serosal surface was allowed to adhere to card for 3 min and was then fixed in 10% neutral buffered formalin for 18 h at 20°C. Paraffin sections (4 μm) were stained with haematoxylin and eosin, and mucosal thickness (stomach) or crypt length (jejunum, ileum, caecum, and colon) was measured by video-image analysis. Intraepithelial lymphocytes are equally distributed in all parts of the small intestine, and are known to increase when non-specific intestinal damage occurs. Thus, to assess potential damage, intraepithelial lymphocytes were counted in eight jejunal villi from each of the six rats fed diets containing GNA-GM potatoes or parent potatoes, both raw and boiled. No such measurements were made for the group fed parent potatoes spiked with GNA because dietary GNA or other lectins do not induce lymphocyte infiltration. GNA binding to the jejunum and ileum was measured by elution with 0·1 mol/L mannose, followed by ELISA.

	Mean (SD) crypt length (μm) and difference between treatments*						Statistical analysis (p)†			Interaction (p)†	
	Parent	Parent *vs* parent+GNA (p)	Parent+GNA	Parent+GNA *vs* GNA-GM (p)	GNA-GM	Parent *vs* GNA-GM (p)	Effect of GNA	Effect of cooking	Effect of trans-formation	GNA×cook	Trans×cook
Stomach											
Boiled	294 (46)	0·29	347 (42)	0·37	339 (36)	0·02	0·001	0·052	0·868	0·917	0·543
Raw	261 (32)	0·03	312 (32)	0·98	323 (54)	0·07					
p	0·18		0·94		0·35						
Jejunum											
Boiled	75 (19)	0·72	78 (17)	0·97	78 (12)	0·71	0·029	0·171	0·041	0·035	0·037
Raw	57 (8)	0·14	64 (11)	0·01	90 (20)	<0·01					
p	0·06		0·09		0·24						
Ileum											
Boiled	59 (8)	0·20	55 (7)	0·12	63 (13)	0·43	0·221	0·001	0·106	0·209	0·942
Raw	71 (9)	0·24	79 (13)	0·43	87 (25)	0·15					
p	0·02		<0·01		0·06						
Caecum											
Boiled	95 (19)	0·90	98 (21)	0·04	70 (15)	0·05	0·033	0·001	0·566	0·497	0·021
Raw	132 (19)	0·02	104 (17)	0·25	119 (25)	0·35					
p	<0·01		0·55		<0·01						
Colon											
Boiled	146 (15)	0·02	177 (24)	0·02	139 (24)	0·65	0·878	0·002	0·181	0·231	0·001
Raw	192 (34)	0·04	148 (25)	<0·01	215 (34)	0·28					
p	0·02		0·07		<0·01						

Data are the means of six animals calculated from five observations for each. GNA×cook=interaction between GNA and cooking; Trans×cook=interaction between transformation and cooking.
*By Student's *t* test. †By multivariate analysis with Tukey's test.

Table 1: **Effect of raw and cooked parent, parent+GNA, and GNA+GM potatoes on histological indices of rat gut**

	Raw potato		Boiled potato	
	Parent+GNA	GNA-GM	Parent+GNA	GNA-GM
GNA intake (μg)	30	29	15	5·6
Mean (SD) bound GNA (μg)				
Jejunum	0·47 (0·28)	0·37 (0·27)	0·25 (0·21)	0·05 (0·04)
Ileum	0·28 (0·15)	0·44 (0·25)	0·17 (0·08)	0·07 (0·02)
Remainder	5·04 (2·67)	2·23 (0·63)	0·78 (0·35)	0·20 (0·17)
Total	5·79 (2·71)	3·04 (0·60)	1·20 (0·49)	0·32 (0·17)

On the morning of day 10, rats were given 1·5 g allocated diet and were killed 2 h later. After dissection, oesophagus, pylorus, and ileocaecal junction were clipped, and small intestine was washed thoroughly with saline. Small intestine was cut into three segments: jejunum (first 20 cm), ileum (last 20 cm), and remainder. Tissues were homogenised with phosphate-buffered saline containing 0·1 mol/L mannose, and solutions were used for determination of GNA content by competitive ELISA.

Table 2: **GNA binding to the jejunum and ileum of rats given diets containing GNA-GM potatoes or parent potato diets spiked with GNA**

The presence of GNA in the diets, irrespective of whether originating from GNA-GM potatoes or from parent-potato diets supplemented with GNA, was associated with significantly greater mucosal thickness of the stomach when compared with parent-potato diets (table 1). This effect was observed with both raw and boiled potatoes. Crypt length in the jejunum of rats fed on raw GNA-GM potato diets was significantly greater than in those given parent-line or parent-line plus GNA potato diets. However, the increase in jejunal crypt length was not seen in rats fed boiled GNA-GM potatoes (table 1). GNA had no significant effects on the ileum, but rats fed boiled potatoes had shorter ileal crypts than rats given respective raw potato diets. Rats fed boiled GNA-GM potatoes had significantly thinner caecal mucosae than rats given boiled parent potatoes, with or without GNA supplementation (table 1). Intraepithelial lymphocyte counts per 48 villi were 7·6 (SD 2·7) in rats fed on boiled parent potatoes, compared with 10·3 (3·3) in rats fed boiled transgenic potatoes ($p<0·01$). With raw potato diets, the intraepithelial lymphocyte counts were again significantly different: 5·3 (2·0) and 9·3 (2·6) in parent and GM potatoes, respectively ($p<0·01$). Peyer's patches appeared normal in all rats. GNA binding in the jejunum and ileum was about the same, irrespective of whether spiked GNA potatoes or GM potatoes were fed (table 2). Measurement of GNA binding by immunocytochemistry also showed a similar pattern.[2]

We suggest that the promotion of jejunal growth was the result of the transformation of the potato with the GNA gene, since the jejunum of rats was shown to be stimulated only by GM potatoes but not by dietary GNA (table 1), in agreement with a previous study in which the dietary GNA concentration was 1000-fold higher than the one used in this study.[2] Thus, we propose that the unexpected proliferative effect was caused by either the expression of other genes of the construct, or by some form of positioning effect in the potato genome caused by GNA gene insertion. Because caecal thickness was similar in rats given boiled parent potatoes in the presence or absence of spiked GNA, we suggest that the decrease in caecal mucosal thickness seen in rats fed boiled GM-potato diets was the consequence of the transfer of the GNA gene into the potato. Caecal mucosal thickness in rats given raw potato diets was significantly higher than in those given the corresponding boiled potatoes. Thus, the main effect of boiling was to decrease mucosal thickness; this binding was fully in line with expectations. The raw parent-line potato diets supplemented with GNA were associated with a significantly thinner caecal mucosa than that of rats given parent-line potato diets. A similar trend was also observed in rats fed raw GNA-GM potatoes, but the difference did not reach significance (table 1).

As expected, colonic crypt lengths were generally higher in rats given raw potato diets than in those given boiled potatoes, except for animals fed GNA-supplemented raw or boiled potato diets, between which there was no significant difference. Feeding rats on diets containing GM potatoes, irrespective of whether raw or boiled, had no significant effect on colonic crypt length compared with that in animals fed the corresponding parent-line potatoes (table 1). Rats fed on GNA-supplemented parent potatoes had significantly shorter colonic crypt lengths than those fed on parent potatoes of GNA-GM potatoes; the reason for this finding is not clear.

In conclusion, the stimulatory effect of GNA-GM potatoes on the stomach was mainly due to the expression of the GNA transgene in the potato. By contrast, the potent proliferative effect of raw GNA-GM potatoes on the jejunum, and the antiproliferative effect of boiled transgenic potatoes on the caecum can be attributed only partly to GNA gene expression. Other parts of the GM construct, or the transformation, could have contributed to the overall effects. Once bound, GNA is internalised by endocytosis;[2] some other component of the construct in the GNA-GM potato or its expressed gene product might also be able to penetrate and affect the rat mucosal cells in a similar manner. The growth-promoting effect of raw GNA-GM potatoes in the jejunum, evident as crypt hyperplasia, is probably due to a direct stimulatory effect on crypt cells; the increase in T lymphocyte infiltration may be important in the elimination of damaged enterocytes.[3] The possibility that a plant vector in common use in some GM plants can affect the mucosa of the gastrointestinal tract and exert powerful biological effects may also apply to GM plants containing similar constructs, particularly those containing lectins, such as soya beans or any plants expressing lectin genes or transgenes.

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Differential binding of the insecticidal lectin GNA to human blood cells

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See Commentaries pages 1312, 1313

Evidence of snowdrop lectin binding to human white cells supports the need for greater understanding of the possible health consequences of incorporating plant lectins into the food chain.

There is interest in the possible use of lectins to protect food plants from attack by insects. Many of these carbohydrate-binding proteins agglutinate vertebrate red blood cells. The lectin peanut agglutinin (PNA) also binds to the Thomsen-Friedenreich antigen on the surfaces of some human colon cells. After eating peanuts, PNA has been detected in the

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RESEARCH ARTICLE

Fine Structural Changes in the Ileum of Mice Fed on δ Endotoxin-Treated Potatoes and Transgenic Potatoes

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ABSTRACT The present work has been designed to study the effect of feeding on transgenic potatoes, which carry the CryI gene of *Bacillus thuringiensis* var. *kurstaki* strain HD1, on the light and electron microscopic structure of the mice ileum, in comparison with feeding on potatoes treated with the 'δ-endotoxin' isolated from the same bacterial strain. The microscopic architecture of the enterocytes of the ileum of both groups of mice revealed certain common features such as the appearance of mitochondria with signs of degeneration and disrupted short microvilli at the luminal surface. However, in the group of mice fed on the 'δ-endotoxin', several villi appeared with an abnormally large number of enterocytes (151.8 in control group versus 197 and 155.8 in endotoxin and transgenic-treated groups, respectively). Fifty percent of these cells were hypertrophied and multinucleated. The mean area of enterocyte was significantly increased (105.3 μm^2 in control group versus 165.4 μm^2 and 116.5 μm^2 in endotoxin and transgenic-treated groups, respectively). Several forms of secondary lysosomes or auotophagic vacuoles were recognized in these cells. These changes were confirmed with the scanning electron microscope which revealed a remarkable increase in the topographic contour of enterocytes (23 μm in control group versus 44 μm and 28 μm in endotoxin and transgenic-treated groups, respectively) at the divulged surface of the villi. The basal lamina along the base of the enterocytes was damaged at several foci. Several disrupted microvilli appeared in association with variable-shaped cytoplasmic fragments. Some of these fragments contained endoplasmic reticulum, as well as ring-shaped annulate lamellae. In addition, the Paneth cells were highly activated and contained a large number of secretory granules. These changes may suggest that δ-endotoxin-treated potatoes resulted in the development of hyperplastic cells in the mice ileum. Although mild changes are reported in the structural configuration of the ileum of mice fed on transgenic potatoes, nevertheless, thorough tests of these new types of genetically engineered crops must be made to avoid the risks before marketing.

Key words: scanning; ultrastructure; ileum; *Bacillus thuringiensis* var. *kurstaki*; transgenic potatoes; δ-endotoxin

INTRODUCTION

This study respects the efforts of several investigators against the dangerous use of chemical insecticides for pest control; these chemicals are still widely marketed (Fares, 1996). In the mid 1970s, the World Health Organization (WHO) and other international institutions initiated studies on the development of existing and new biological control agents (de Barjac, 1989). The most popular of these agents are strains of the '*Bacillus thuringiensis*'. Among these *Bacillus thuringiensis* var. *kurstaki*, was proven to produce an effective toxin against lepidopteran insects (Tyrell *et al.*, 1981, de Barjac, 1989; Singsit *et al.*, 1997). These spore-forming entomopathogenic bacteria are gram-positive and have a unique ability to produce parasporal-proteinaceous crystalline inclusions during sporulation (Caramori *et al.*, 1991; Sanchis *et al.*, 1996). The insecticidal properties of this protein crystal (δ-endotoxin) have stimulated studies leading to its commercial production for use as a biological control agent (Sanchis *et al.*, 1996). Scientists at AGERI

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Figure 1. Scanning electron micrograph of the intestinal mucosa of control group revealing the luminal surface of the villi covered by enterocytes (E) and occasional small pits indicating the sites of mucous cells (m). Bar = 10 μm

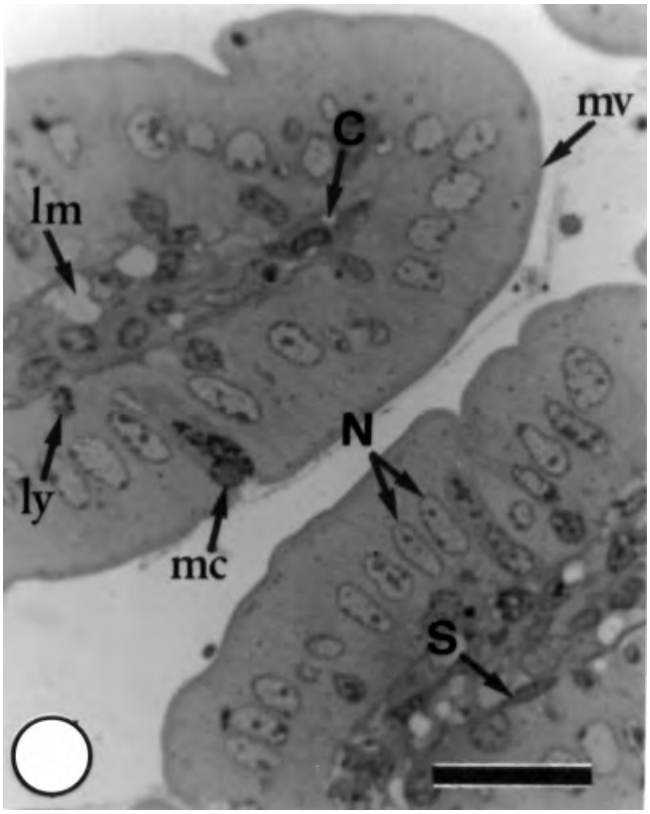

Figure 2. Semithin section of the intestinal villi of the control group revealing the enterocytes with typical oval nuclei (N) and a continuous thin ribbon of tightly packed microvilli (mv), mucous cells (mc), with their dark mucin granules, intraepithelial lymphocytes (ly), blood capillaries (C), lymphatics (lm) and smooth muscles (S). Bar = 20 μm

(Agriculture Genetic Engineering Research Institute, Guiza, Egypt) were able to produce transgenic potatoes in which the CryI gene of *Bacillus thuringiensis* var. *kurstaki* was transmitted into the plant cells via a shuttle plasmid vector after cloning in *E. coli*. The present investigation has been designed to evaluate feeding of experimental animals on 'transgenic potatoes' (as yet not measured) on the ileum of mice at the microscopic level, compared with feeding on potatoes treated with the bacterial toxin 'δ-endotoxin'.

MATERIALS AND METHODS

Preparation of Bacterial Endotoxin

Bacterial isolates of the strain HD14 of *Bacillus thuringiensis* var. *kurstaki* were allowed to grow in sterilized T3 medium (5.0 g peptone, 1.5 g yeast extract, 0.005 g Mn Cl_2 and 0.5 M sodium phosphate buffer at pH 6.8) according to Travers *et al.* (1987). Sporulation was examined at intervals using a light microscope. Bacterial spores and crystals were collected using a Backman J-2MC centrifuge equipped with a JA-14 titanium rotator at 1200 rev min^{-1} for 20 min at 4 °C. Sedimented spores and crystals (δ-endotoxin) were washed in distilled water and dried for 6 h (under vacuum) in 'Labconco, Freeze Dry/Shell Freeze' system (model ilyph, lock 6) according

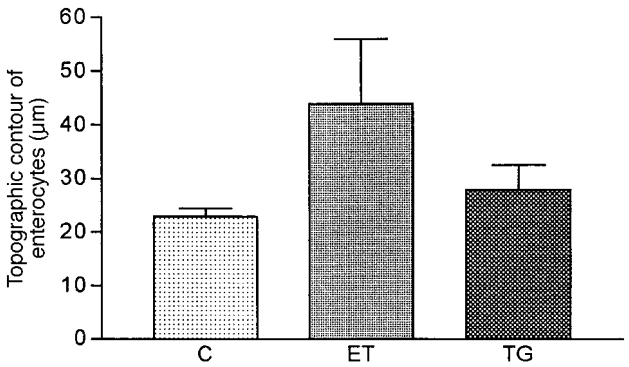

Figure 3. Variation in the topographic control of enterocytes in in SEM images of ileum for each of the three groups of mice: control group (C), δ-endotoxin-treated group (ET) and transgenic potatoes-treated group (TG). Bars denote the standard deviation in each group

Table 1. Statistical analysis of the mean perimeter (topographic contour) of enterocyte in scanning electron microscopic images of ileum for each of the three different groups of mice: control group (C), δ-endotoxin-treated group (ET), and transgenic potatoes-treated group (TG)

	Control (C)	Endotoxin (ET)	Transgenic (TG)
Number of measured cells	50	50	50
Mean perimeter of cell in 5 mice	**23.00**	**44.00**	**28.00**
Minimum perimeter of cell	21.00	30.00	22.00
Median perimeter of cell	24.00	44.00	30.00
Maximum perimeter of cell	24.00	58.00	32.00
Standard deviation	1.477	11.94	4.513
Standard error	0.4264	3.447	1.303
p value (two-tailed)	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Significant (alpha = 0.05)?	Yes	Yes	Yes
Unpaired t-test:	Two-tailed p value:		
1: C vs ET	p value <0.0001 (means are significantly different, $p < 0.05$)		
2: C vs TG	p value = 0.0014 (means are significantly different, $p < 0.05$)		

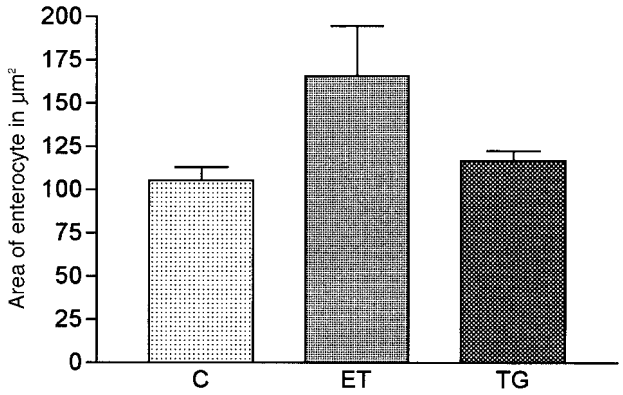

Figure 4. Variation in the area of enserocytes in semithin sections of ileum of each of the three different groups of mice: control group (C), δ-endotoxin-treated group (ET) and transgenic potatoes-treated group (TG). Bars denote the standard deviation in each group

to Redway and Lapage (1974). The dried δ-endotoxin was stored at 20 °C. Fresh potatoes were cut into small pieces and immersed in a suspension of the δ-endotoxin, of *Bacillus thuringiensis* var. *kurstaki*, in distilled water (1 g l ^{1}) for 30 min.

Feeding of Mice

A group of 5 1-month-old male mice (*Mus musculus*), was fed daily for 2 weeks on a diet consisting of the δ-endotoxin-treated potatoes. Another group of 5 mice was fed on a diet consisting of transgenic potatoes, carrying the CryI gene of *Bacillus thuringiensis* var. *kurstaki*, for 2 weeks. These transgenic plants were provided by AGERI (Guiza, Egypt). A control group of 5 mice was fed on fresh potatoes for the same 2-week period.

Table 2. Statistical analysis of the mean area of enterocyte in semithin sections of ileum of each of the three different groups of mice. control group (C), δ-endotoxin-treated group (ET), and transgenic potatoes-treated group (TG)

	Control (C)	Endotoxin (ET)	Transgenic (TG)
Number of measured cells	750	750	750
Mean area of cell in 5 mice	**105.3**	**165.4**	**116.5**
Minimum area of cell	99.00	125.0	111.0
Median area of cell	103.5	172.0	115.0
Maximum area of cell	115.0	192.5	125.0
Standard deviation	7.762	28.70	5.972
Standard error	3.881	14.35	2.986
p value (two tailed)	$p < 0.0001$	$p < 0.0014$	$p < 0.0001$
Significant (alpha = 0.05)?	Yes	Yes	Yes
Unpaired t-test:	Two-tailed p value:		
1: C vs ET	p value = 0.0068 (means are significantly different, $p < 0.05$)		
2: C vs TG	p value = 0.06 (means are not significantly different, $p < 0.05$)		

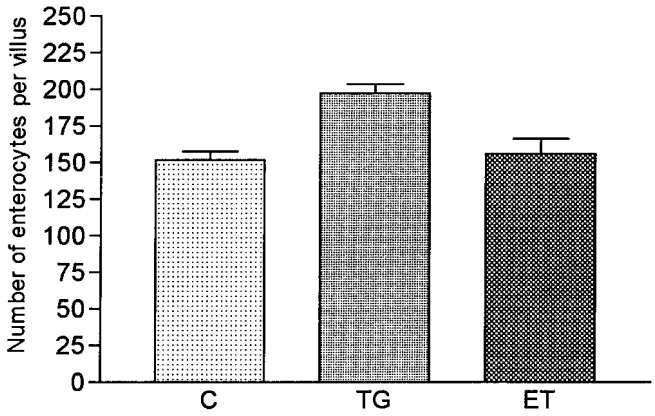

Figure 5. Variation in number of enterocytes pervillus in semithin sections of ileum of each of the three different groups of mice: control group (C), δ-endotoxin-treated group (ET) and transgenic potatoes-treated group (TG), Bars denote the standard deviation in each group

Table 3. Statistical analysis of the mean number of enterocytes in semithin sections of ileum of each of the three different groups of mice: control group (C), δ-endotoxin-treated group (ET), and transgenic potatoes-treated group (TG)

	Control (C)	Endotoxin (ET)	Transgenic (TG)
Number of villi selected in 5 mice	625	625	625
Mean number of cells per villus (in 5 mice)	**151.8**	**197.0**	**155.8**
Minimum number of cells	148.0	190.0	140.0
Median number of cells	149.5	196.5	160.5
Maximum number of cells	160.0	205.0	162.0
Standard deviation	5.560	6.164	10.53
Standard error	2.780	3.082	5.266
p value (two tailed)	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Significant (alpha = 0.05)?	Yes	Yes	Yes
Unpaired *t*-test:	Two-tailed *p* value:		
1: C vs ET	*p* value < 0.0001 (means are significantly different, $p < 0.05$)		
2: C vs TG	*p* value = 0.5268 (means are not significantly different, $p < 0.05$)		

Preparation of Microscopic Samples

Animals from the three different groups were killed by severing the spinal cord and the ileum of each animal was dissected out, cut into small pieces and fixed in 2.5 % glutaraldehyde in 0.l M phosphate buffer (Sigma, St Louis, USA) at pH 7.2 for 90 min, for light, scanning and electron microscopic studies. Tissues were postfixed for 2 h in 1 % OsO^4 in the same phosphate buffer, dehydrated through ascending grades of acetone and embedded in Spurr's medium. Semithin sections (0.5 µm) were prepared on an MT600-XL RMC ultratome (Tokyo, Japan), stained with toluidine blue and used for light microscopic studies. Thin sections (80–90 nm) were cut with a Diatom diamond knife (Washington, USA) on an MT600-XL RMC ultratome (Tokyo, Japan). Sections were collected on 200-mesh nickel grids, stained in 5% uranyl acetate in distilled water for 10 min, washed in distilled water and stained in lead citrate for 6 min (Venable and Coggeshall, 1965) and examined with a bi-functional Joel JTM-1200 EX II electron microscope (Tokyo, Japan). For scanning electron microscopic studies, small pieces of the OsO_4-postfixed tissues were exposed to the critical point dry and spotter coating processes and examined by the same electron microscope.

Morphometric Analysis

Semithin sections, as well as scanning and electron microscopic photographs, of the ileum of each of the three different groups of mice were used in the morphometric studies. Images of the ileum from these preparations were transferred into an IBM computer

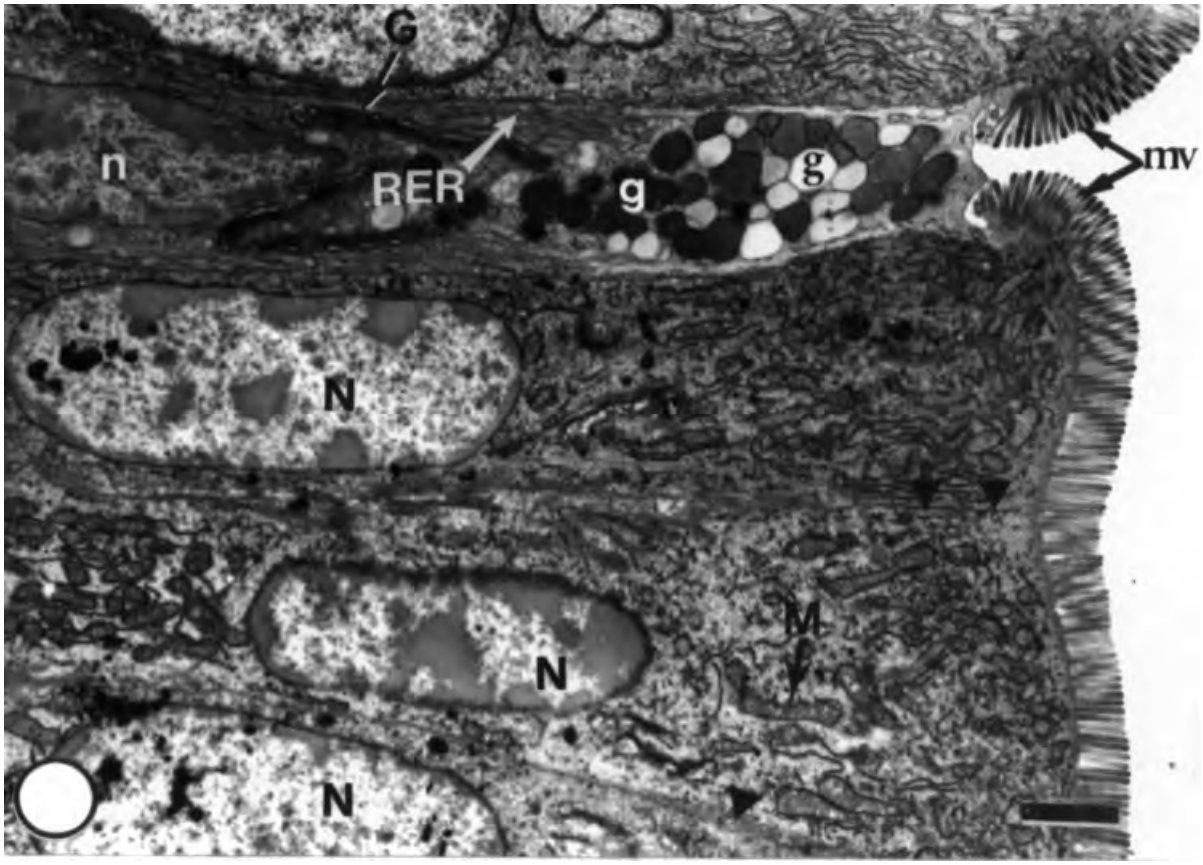

Figure 6. Electron micrograph of the intestinal epithelium of control group revealing a mucous cell with apical mucin globules (g), basal flattened nucleus (n), rough endoplasmic reticulum (RER) and Golgi complex (G). The enterocytes display luminal microvilli (mv), large oval euchromatic nuclei (N), tight junction (arrows), rough endoplasmic reticulum (arrow heads) and mitochondria (M). Bar = 2.0 μm

attached to an Olympus™ light microscope (Japan) via a Sony™ video-camera (Japan). The captured images were then digitized on the computer using an 'Alpha-Viewer' image analysis program, version 1.0, for measuring the topographic contour and the area of enterocytes. The number of enterocytes, multinucleated enterocytes, and hypertrophied nuclei were also counted. The mean value of each parameter was calculated per 5 animals, in each of the three different groups, and the data were statistically analysed using Paired Student's *t*-test of the 'GraphPad Prism™' program, version 2.01, from Graph-Pad Software Inc., USA.

OBSERVATION

Microscopic Observations

In relation to the digestive and absorptive functions of the small intestine of mammals, the mucosa of the ileum is the most important absorptive layer (Fawcett, 1997). Accordingly, the present investigation was designed to focus mainly on the microscopic structure of this layer in mice of the three different groups: the control group, the group fed on the δ-endotoxin-treated potatoes and the group fed on transgenic potatoes.

Control Group

As revealed by the scanning electron microscopic examination, the intestinal mucosa was thrown up into several finger-like, as well as leaf-like forms of villi extending into the intestinal lumen (Figure 1). The surface of these villi was almost entirely covered by the enterocytes, which were the principle absorptive cells of the intestinal epithelium. The topographic contour (mean perimeter) of the enterocyte was 23 μm, $p < 0.0001$ (Table 1, Figure 3). Scattered among the enterocytes were occasional small pits indicating the sites of mucous cells. The light microscopic examination of semithin sections of these villi revealed the enterocyte as a tall columnar cell with typical oval nuclei in the lower third of the cell (Figure 2). The mean area of the enterocyte was 105.3 μm^2, $p < 0.0001$ (Table 2, Figure 4), while the mean number per one villus was 151.8, $p < 0.0001$

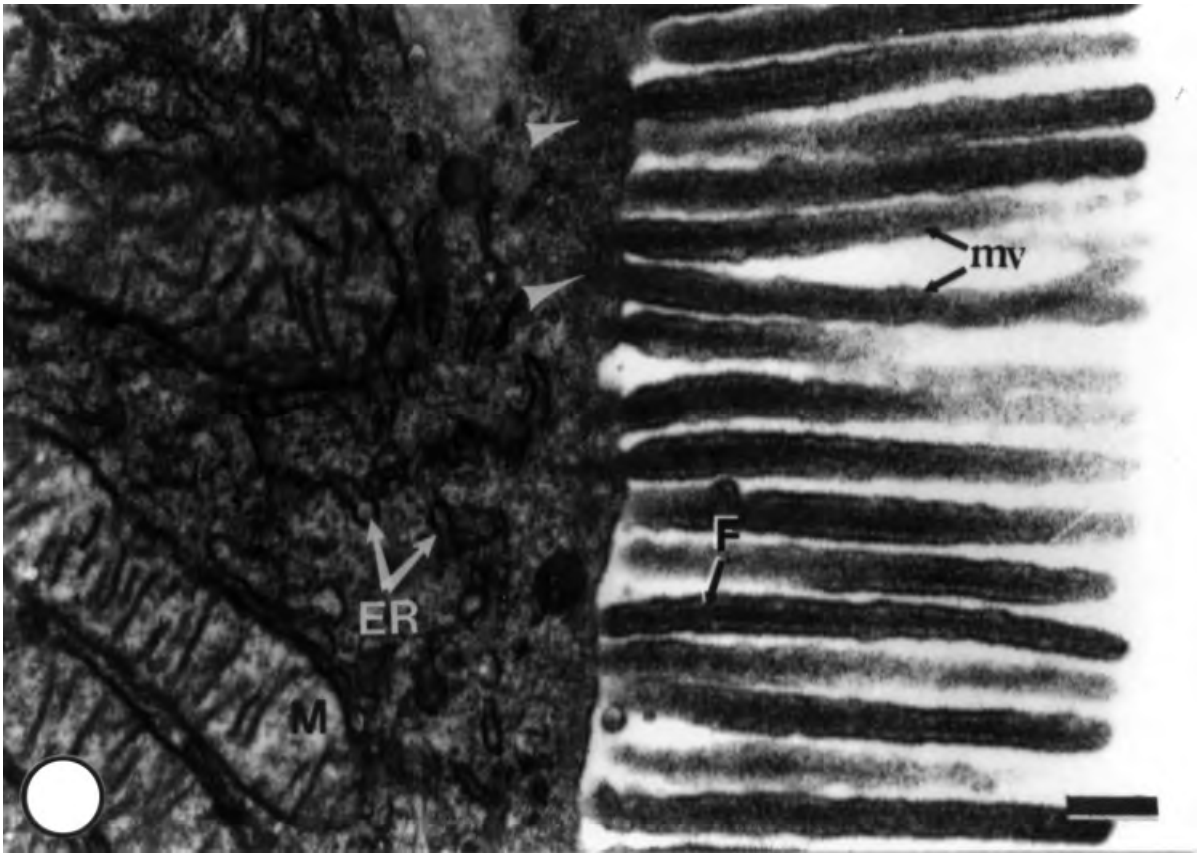

Figure 7. Electron micrograph of the intestinal epithelium of control group revealing a part of an enterocyte with relatively tall mitochondria (M), several profiles of endoplasmic reticulum (ER) and a large numbers of closely packed parallel microvilli (mv). Each microvillus has a bundle of thin striated filaments (F) connected to terminal web (arrow heads) in a clear zone of underlying cytoplasm. Bar = 0.2 μm

(Table 3, Figure 5). The luminal surface of these cells was covered by a continuous thin ribbon, which was a highly specialized region of this epithelium, consisting of tightly packed microvilli. Mucous cells were located among the enterocytes and were distinguished by their mucin granules which occupy the upper portion of the cells. Their nuclei were small in size and oval in shape and were located at the basal side of the cells. Intraepithelial lymphocytes were located in a basal position between the lateral intercellular spaces. They possessed relatively small dark nuclei. Underneath the basal lamina of the intestinal epithelium, the lamina propria penetrated the core of the villi, taking along blood capillaries, lymphatics and smooth muscles.

At the ultrastructural level, the mucous cells were recognized by their mucin globules (Figure 6). These droplets occupied the apical region of the cell and consisted of a homogeneous matrix, which varied in intensity from highly electron dense to more lightly electron dense, enveloped by a delicate membrane. The base of the cell was relatively free of secretory material and formed a slender stem or stalk. The nucleus tended to be flattened and was surrounded by a thin layer of cytoplasm. This cytoplasmic area contained several profiles of longitudinally oriented rough endoplasmic reticulum running parallel to the lateral edges of the cell. A highly developed Golgi complex was situated between the nucleus and the mucin droplets.

The enterocytes displayed large oval euchromatic nuclei with a few patches of heterochromatin (Figure 6). The lateral walls of these cells formed a well-developed tight junction, specially at the uppermost region. The upper cytoplasmic region was rich in rough endoplasmic reticulum and mitochondria.

The mitochondria were relatively large and had an internal structure with several large cristae traversing across the inner mitochondrial space (Figure 7). The striated or brush border of the enterocytes was made up of large numbers of closely packed parallel microvilli (Figures 6 and 7). Each microvillus was a cylindrical protrusion of the apical cytoplasm and consisted of a cell membrane enclosing a filamentous core. In the interior of

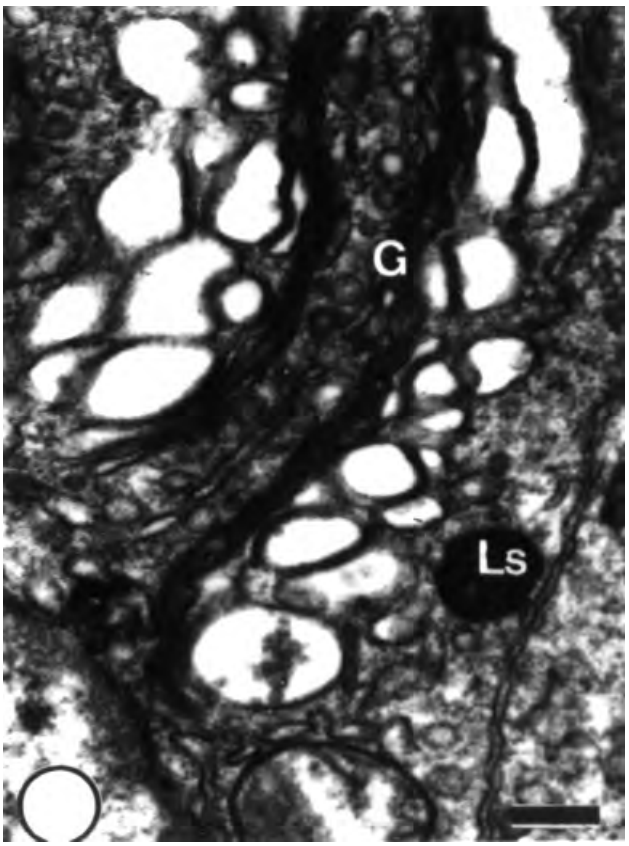

Figure 8. Electron micrograph of the intestinal epithelium of control group revealing a part of an enterocyte with well-developed Golgi apparatus (G) and a primary lysosome (Ls). Bar = 0.2 μm

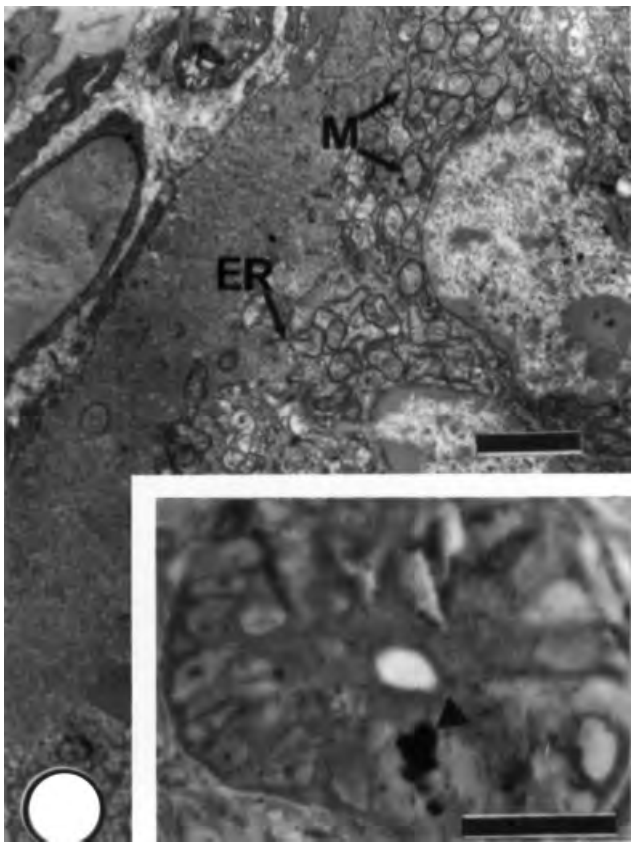

Figure 9. Electron micrograph of a basal region of intestinal epithelium of a control mouse revealing a large number of subnuclear mitochondria (M), a few profiles of endoplasmic reticulum (ER) and a thick basal lamina (asterisk). Bar = 2.0 μm. The inset reveals a semithin section of a part of the crypt of Lieberkühn containing a few Paneth cells with dark secretory granules (arrowhead). Bar = 20 μm

each microvillus was a bundle of thin striated filaments of running longitudinally in an otherwise homogeneous fine-textured cytoplasmic matrix (Figure 7). Underneath the microvilli was a clear zone usually devoid of organelles, except for a few profiles of endoplasmic reticulum, but occupied by filamentous striations, or terminal web, parallel to the apical surface of the cell (Figure 7).

Several well-developed Golgi apparatuses occupied a supranuclear position and consisted of parallel cisternae and large vesicles (Figure 8). A few primary lysosomes were located in the area of Golgi apparatus (Figure 8). The subnuclear cytoplasmic area was occupied by a large number of mitochondria and a few profiles of endoplasmic reticulum (Figure 9). The base of the enterocytes was based on a thick basal lamina (Figure 9). A small number of Paneth cells were recognized in the lower third of the crypts of Lieberkühn by their characteristic basal nuclei and secretory granules in their luminal surface (inset, Figure 9).

δ-Endotoxin-Treated Group

In the group of mice fed on the δ-endotoxin-treated potatoes, the scanning electron microscopic examination revealed a remarkable increase in the topographic contour of the enterocytes at the divulged surface of the villi (Figure 10). The mean perimeter of the enterocyte was 44 μm, $p < 0.0001$ (Table 1, Figure 2). In addition, several variable-shaped structures, ranging from round to elongate, were recognized adhering to these villi. In semithin sections, the villi appeared with an abnormally large number of enterocytes and consequently were extremely large (Figure 11). The mean number of enterocytes per villus was 197, $p < 0.0001$ (Table 3, Figure 5), while the mean area of enterocytes was 165.4 μm^2, $p < 0.0014$ (Table 2, Figure 4). A large number (50 %) of the enterocytes in these villi were multinucleated. The great majority of these nuclei were hypertrophied and acquired a round shape, rather than the oval appearance revealed in the enterocytes of the control group. At the ultrastructural level, the nuclei of the enterocytes displayed a typical rounded configuration

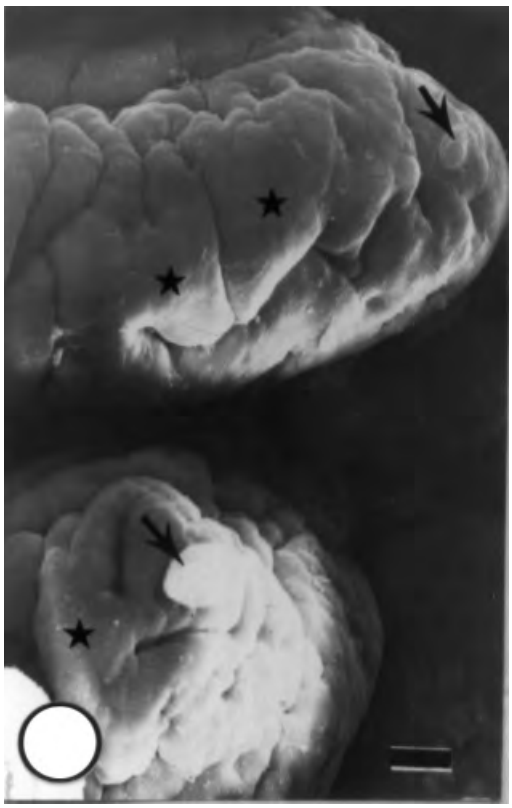

Figure 10. Scanning electron micrograph of the intestinal mucosa of δ-endotoxin-treated group revealing remarkably increased topographic contour of enterocytes (asterisks) and associated variable-shaped structures (arrows). Bar = 10μm

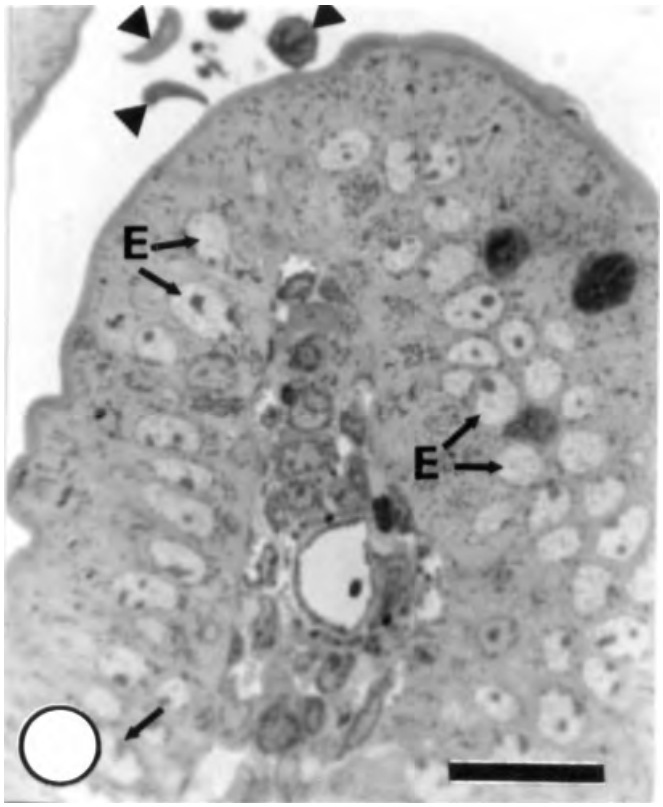

Figure 11. Semithin section of the intestinal mucosa of δ-endotoxin-treated group revealing a villus with an abnormally large number of multinucleated and hypertrophied enterocytes (E). A number variable-shaped cytoplasmic fragments (arrowheads) are in association with this villus. Bar = 20 μm

(Figures 11 and 12). In addition, the basal lamina along the base of the enterocytes was severely destructive at several foci. A number of enterocytes lost their luminal microvilli and appeared in association with variable-shaped cytoplasmic fragments (Figure 12). The rounded forms of these fragments contained several unrecognizable membranous structures, while the elongated forms contained several profiles of endoplasmic reticulum, as well as ring-shaped annulate lamellae (Figures 12 and 13). At one side, these cytoplasmic fragments possessed clear zones which extended laterally into vermiform processes (Figure 13). Most of these cytoplasmic fragments were in association with much smaller rounded structures which were remarkable for their highly electron dense contour and lightly dense core. The lateral plasma membranes of the enterocytes were detached in a number of foci (Figure 14). Their supranuclear cytoplasmic area contained several profiles of endoplasmic reticulum, a few mitochondria and several forms of secondary lysosomes, or auotophagic vacuoles (Figures 14 and 15). Several degenerated mitochondria, as well as endoplasmic reticulum, were located within the autophagic vacuoles (Figure 15). The luminal surface of the enterocytes were covered by short microvilli. The mucous cells in these villi contained several coagulated mucin granules (Figure 16 and inset). In the crypts of Lieberkühn, the Paneth cells were highly activated and contained large number of secretory granules (Figure 17).

Transgenic Potatoes-treated Group

In the group of mice fed on transgenic potatoes, both scanning and light microscopic architecture of the intestinal villi and their cellular structures, including enterocytes, Paneth cells, and mucous cells were almost as normal as the control group (Figures 18–20). The mean perimeter of enterocyte was 28 μm ($p < 0.0001$, Table 1, Figure 2), with a mean area of 116.5 ($p < 0.0001$, Table 2, Figure 4) and a mean number of 155.8 enterocytes per villus ($p < 0.0001$, Table 3, Figure 5). However, at the ultrastructural level the enterocytes possessed several dilated mitochondria with short cristae (Figure 21). In addition, the luminal surface of certain foci possessed disrupted short microvilli. Nevertheless, in the great majority of the enterocytes the microvilli displayed

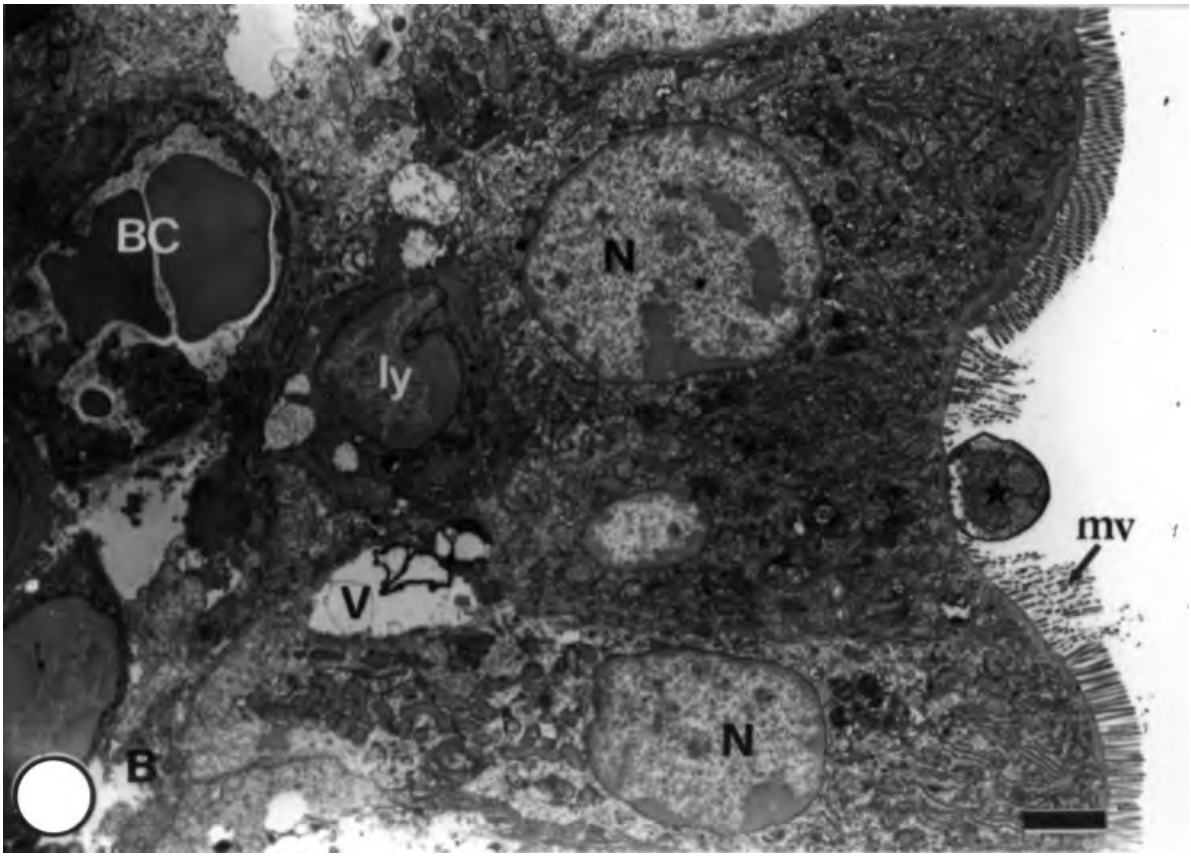

Figure 12. Electron micrograph of the intestinal mucosa of δ-endotoxin-treated group revealing enterocytes with rounded nuclei (N), intracellular vacuoles (V) and discontinuous basal lamina (B) interrupted by a lymphocyte (ly) and congested blood capillary (BC). A cytoplasmic fragment (asterisk) enclosing membranous structures is in association with fragmented microvilli (mv). Bar = 2.0 μm

regular striated appearance (Figure 22). The basal lamina was relatively intact (Figure 18). Mucous cells possessed a homogeneously electron dense mucin granules (Figure 20).

DISCUSSION

In the present investigation, the enterocytes of the intestinal epithelium in the group of mice fed on δ-endotoxin-treated potatoes were remarkably enlarged as a result of multiplication and hypertrophy of their nuclei, degeneration of mitochondria and endoplasmic reticulum, and the ensuing appearance of autophagic vacuoles. These features were reflected on the scanning topographic architecture of these cells, which showed a remarkably large contour. In addition, these changes were accompanied by the detachment of the lateral plasma membranes in several foci and the discontinuation of the basal lamina of these cells. Several investigations revealed that solubilized δ-endotoxin of *Bacillus thuringiensis kurstaki* is cytolytic to a wide range of vertebrate and invertebrate cells (Wu and Chang, 1985; Ibarra and Federici, 1986b; Chilcott and Ellar, 1988). Additionally, Thomas and Ellar (1983a) showed that solubilized endotoxin preparations are lethal when injected into suckling mice. It has been suggested that the high toxicity of this endotoxin is due not to a single protein, but rather to a set of nergistic interactions of the 25-kDa protein with one or more of the higher molecular weight proteins (Chilcott and Ellar, 1988). Although the precise mode of action of the δ-endotoxin of *Bacillus thuringiensis* var. *kurstaki* is not fully understood, Lüthy and Ebersold, (1981) suggested that intoxication in insects may result from an osmotic imbalance across the midgut epithelial membranes which leads quickly to hypertrophy and lysis of midgut cells. Lysis is followed by disruption of the basement membrane, leakage of digestive juices into the hemocoel, and larval death. Thomas and Eliar (1983b) provided good evidence that *Bacillus thuringiensis kurstaki* endotoxin's cytolytic activity was due to a detergent-like action in which the toxin disrupted membranes by binding to specific lipids.

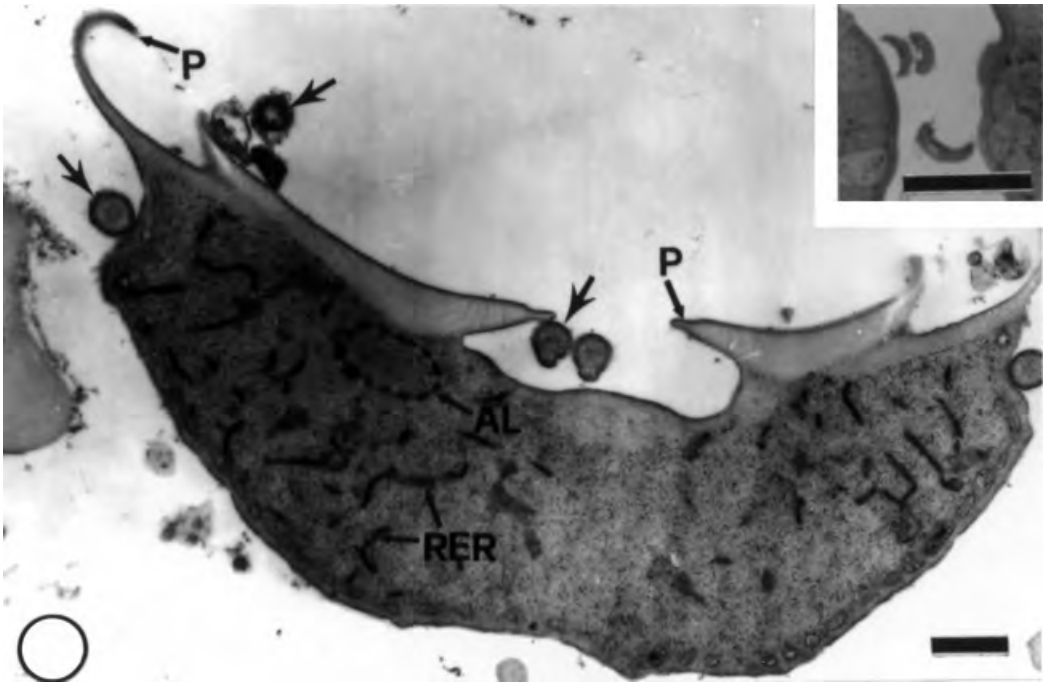

Figure 13. Electron micrograph of the intestinal mucosa of δ-endotoxin-treated group showing an elongated form of cytoplasmic fragments containing several profiles of endoplasmic reticulum (RER), ring-shaped annulate lamellae (AL), clear zones of laterally extended vermiform processes (P), and in association with small rounded structures (arrows) with highly electron dense contour and lightly dense core. Bar = 0.5 μm. Inset: a semithin section of the same area. Bar = 20 μm

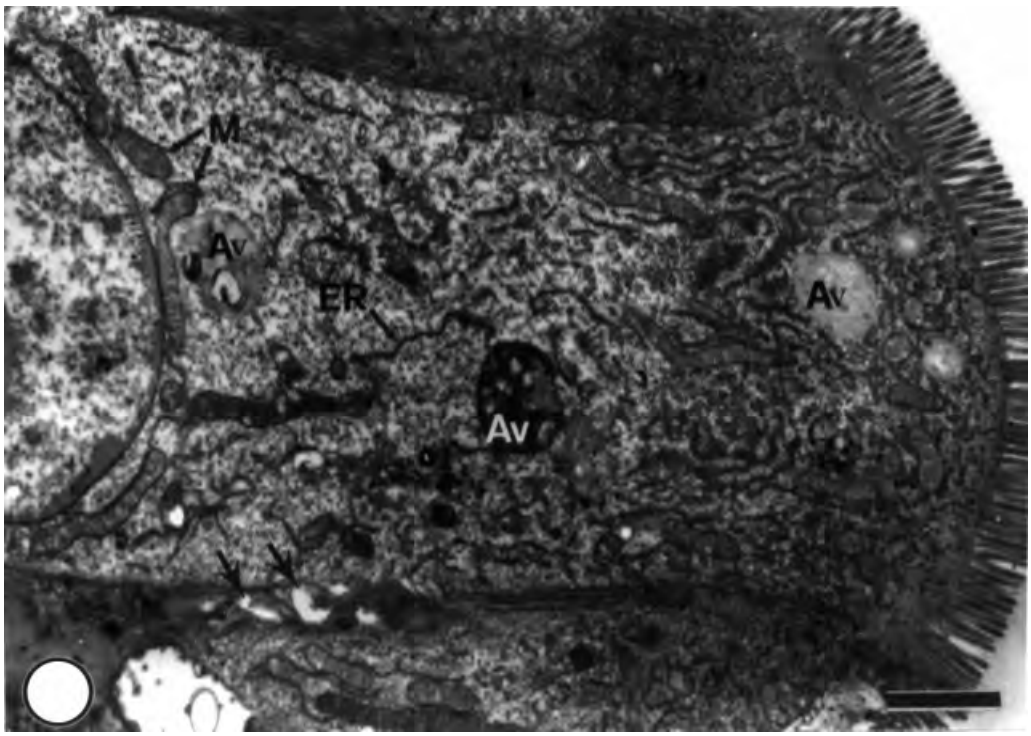

Figure 14. Electron micrograph of the intestinal mucosa of δ-endotoxin-treated group showing detached lateral plasma membranes (arrows), several profiles of endoplasmic reticulum (ER), auotophagic vacuoles (Av), a few mitochondria (M) and a cytoplasmic vacuole. Bar = 2.0 μm

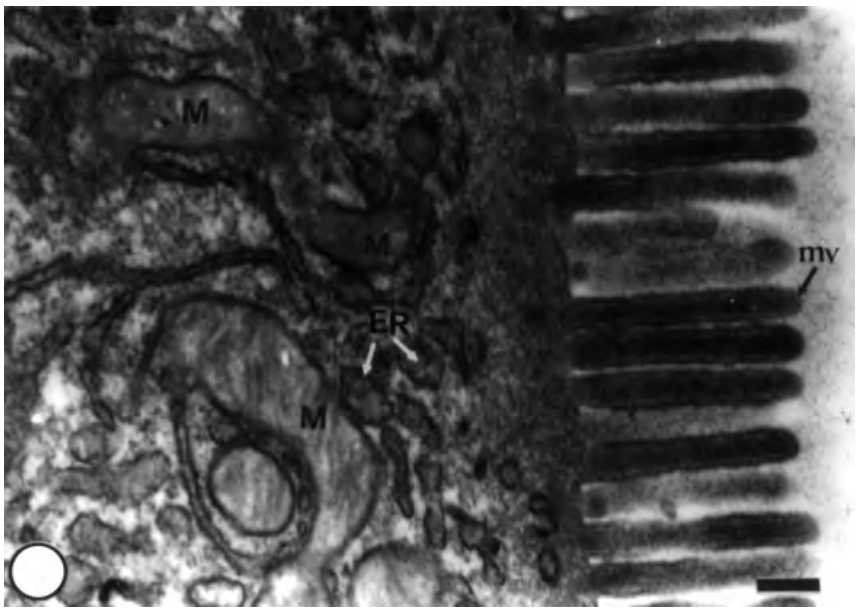

Figure 15. High power electron micrograph of the intestinal mucosa of δ-endotoxin-treated group showing an enterocyte with degenerated forms of mitochondria (M), endoplasmic reticulum (ER) and short microvilli (mv). Bar = 0.2 μm

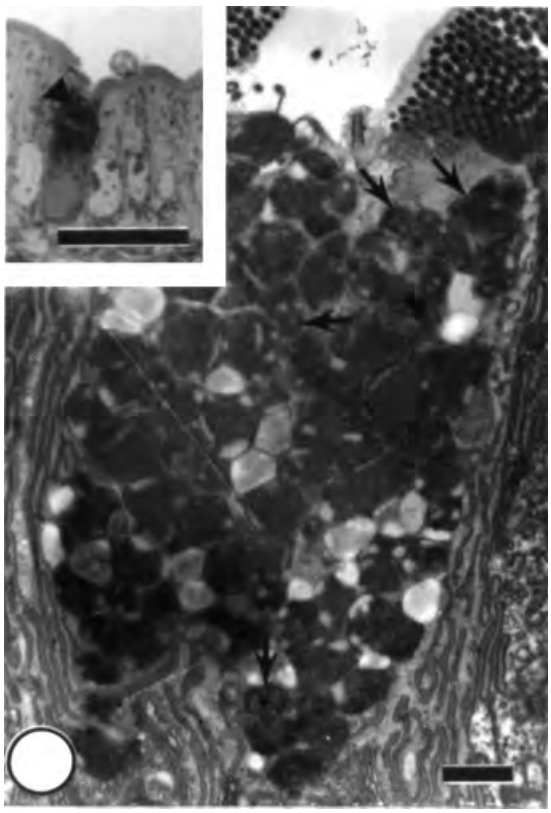

Figure 16. High power electron micrograph of the intestinal mucosa of δ-endotoxin-treated group showing a mucous cell containing several coagulated mucin granules (arrows). Bar = 1.0 μm. Inset: res a mucous cell (arrowhead) in a semithin section of similar area. Bar = 20 μm

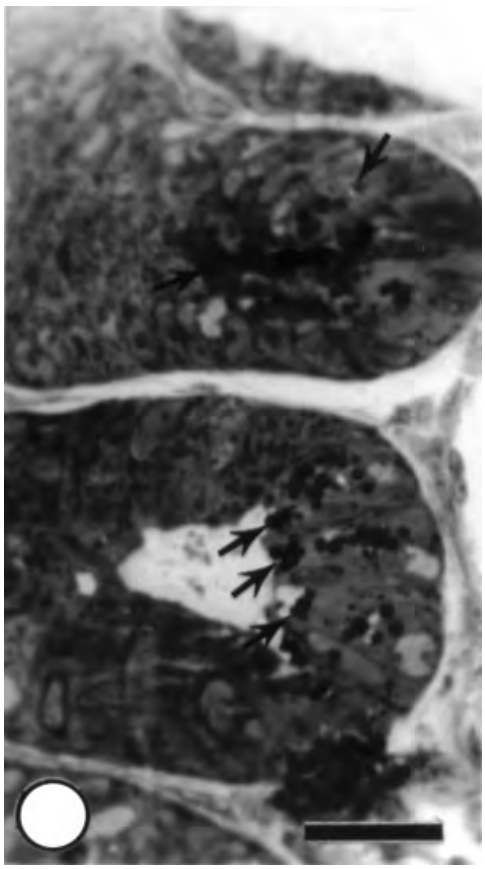

Figure 17. Semithin section of the intestinal mucosa of δ-endotoxin-treated group showing crypts of Lieberkühn with highly activated Paneth cells (arrows). Bar = 20 μm

Figure 18. Scanning electron micrograph of the intestinal mucosa of transgenic potatoes-fed group revealing normal topographic configuration of the enterocytes (asterisks) of the intestinal villi (arrows). Bar = 10 μm

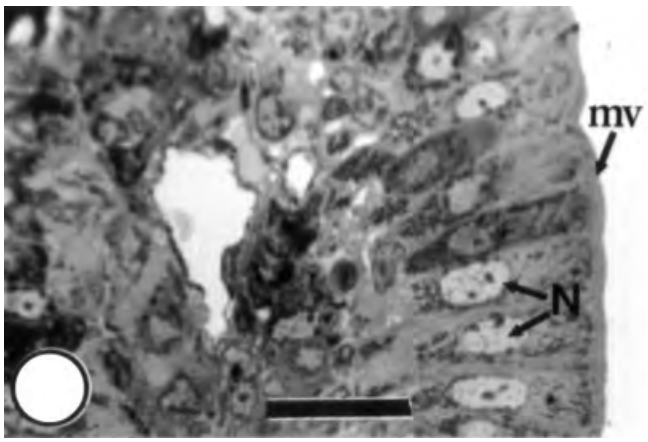

Figure 19. Semithin section of the intestinal mucosa of transgenic potatoes-fed group revealing enterocytes with normal elongated nuclei (N) and normally organized microvilli (mv). Bar = 20 μm

They postulated that the 27 kDa protein was the toxin responsible for cytolytic activity, and acted by binding to the fatty acids phosphatidyl choline and sphingomyelin, among others, as long as these contained unsaturated acyl residues.

In the present investigation, the absence of luminal microvilli in several foci and their association with variable-shaped cytoplasmic fragments in certain other areas may provide a strong evidence of the cytolytic action of the δ-endotoxin of *Bacillus thuringiensis kurstaki* on the intestinal lining epithelium of mice. Some of these fragments contained several profiles of endoplasmic reticulum, as well as ring-shaped annulate lamellae. The presence of annulate lamellae in these cytoplasmic fragments may indicate that they were parts of hyperplastic cells, since several studies revealed the presence of these unique structures in carcinoma cell lines and malignant tumors (Goodlad and Fletcher, 1991; Mirejovsky, 1991; Ueda *et al.*, 1991; Wang *et al.*, 1992; Pettinato *et al.*, 1993). These lamellae are considered to be derived from the outer lamellae of the nuclear membranes or from the intracytoplasmic endoplasmic reticulum (Johannessen, 1979). They are mainly found in rapidly proliferating cell systems, since they may have a role in the nucleocytoplasmic exchange of substances necessary for accelerated protein synthesis, especially in hyperplastic cells. In addition, these fragments were associated with much smaller and rounded structures. These small structures were similar to parasporal bodies of the *Bacillus thuringiensis kurstaki* in their highly electron dense contour and lightly dense core, as previously described by several investigators (Insell and Fitz-James, 1985; Lee *et al.*, 1985; Ibarra and Federici, 1986a). Immunological studies showed that δ-endotoxin of *Bacillus thuringiensis* interacts with the microvilli of the midgut epithelial cells of insects (Ravoahangimalala *et al.*, 1993; Aranda *et al.*, 1996).

In the present investigation a few common features, including mitochondria with signs of degeneration and disrupted short microvilli, were recognized in the

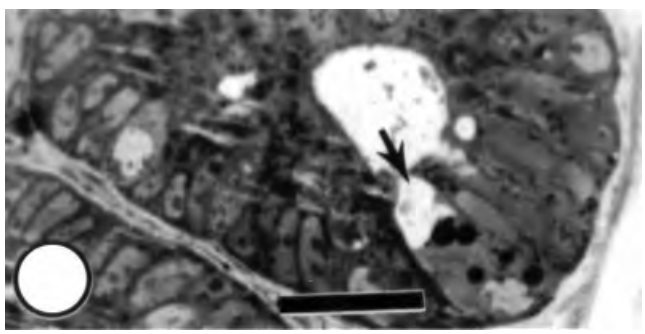

Figure 20. Semithin section of a part of the intestinal mucosa of transgenic potatoes-fed group revealing Paneth cells, with a few secretory granules (arrow), within the crypts of Lieberkühn. Bar = 20 μm

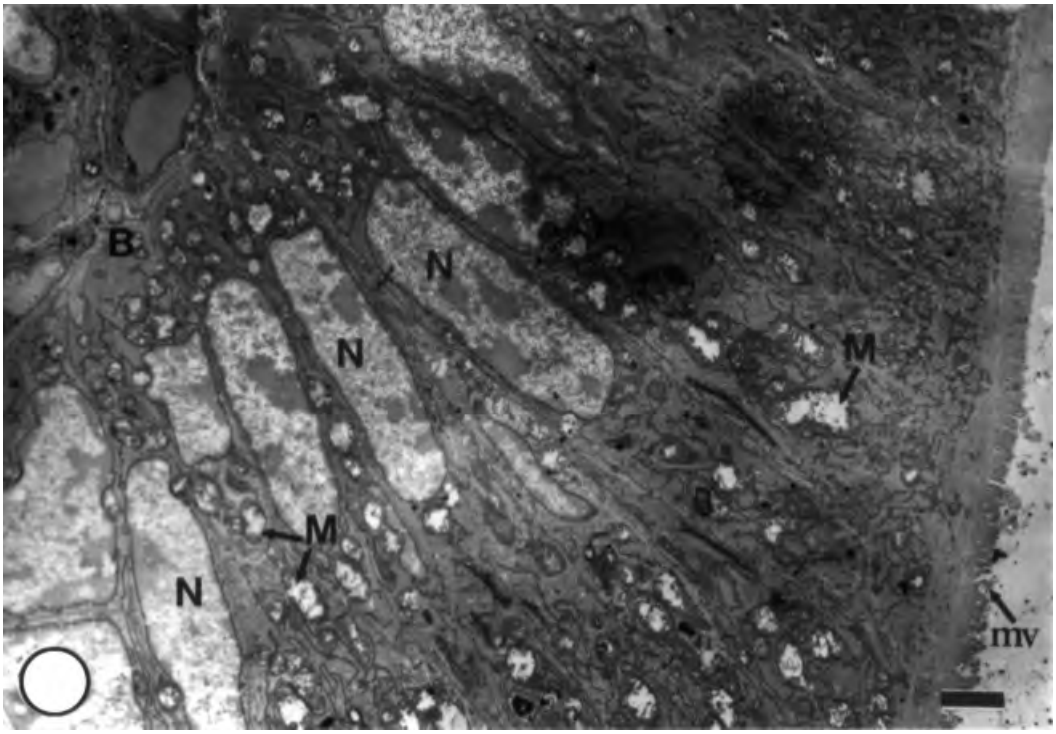

Figure 21. Electron micrograph of the intestinal mucosa of transgenic potatoes-fed group revealing enterocytes with elongated nuclei (N), a relatively intact basal lamina (B) and several dilated mitochondria with short cristae (M). The luminal surface of certain foci possessed disrupted short microvilli (mv). Bar = 2.0 μm

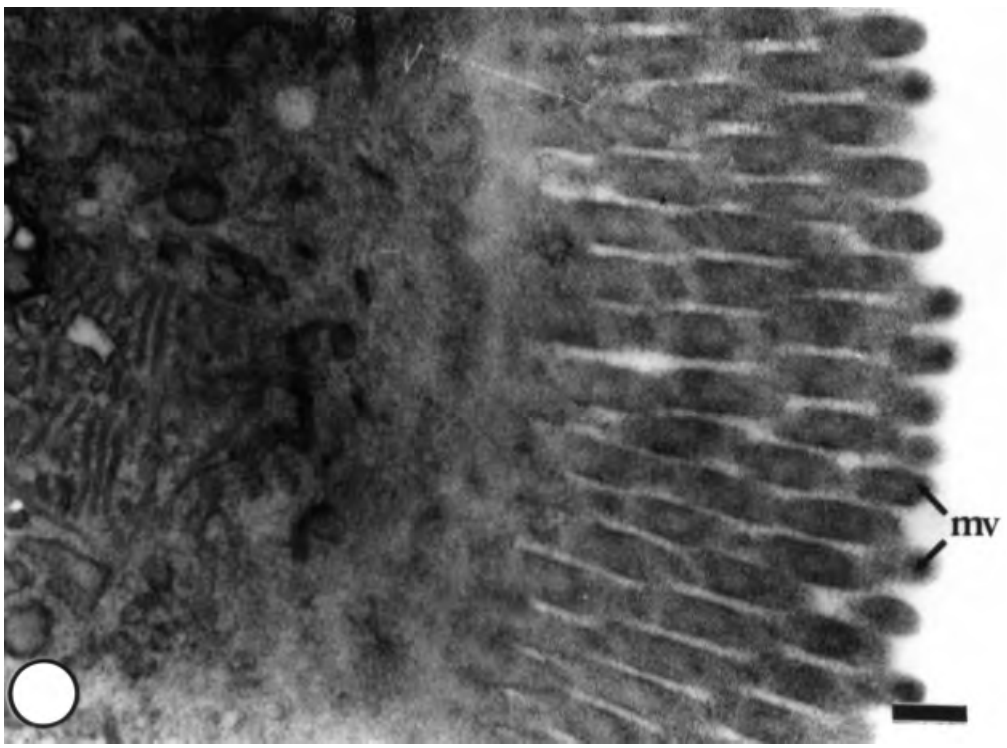

Figure 22. High power electron micrograph of the intestinal mucosa of transgenic potatoes-fed group revealing normally organized microvilli (mv). Bar = 0.2 μm

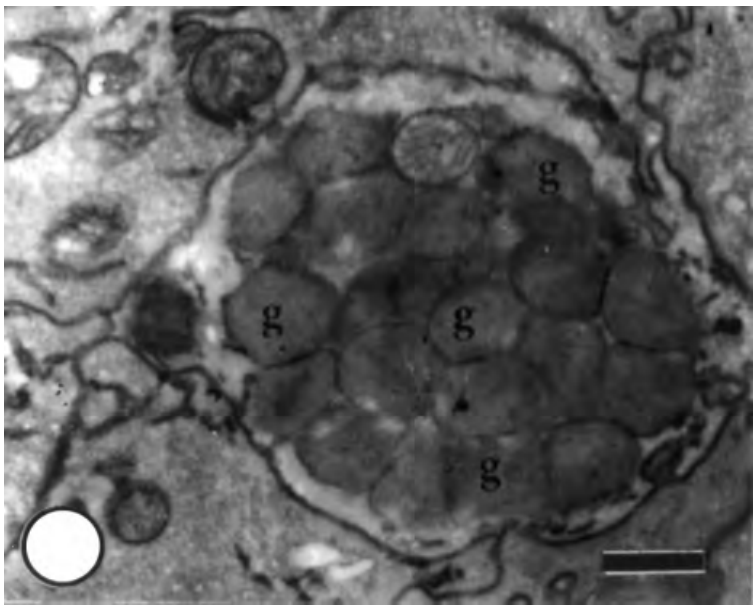

Figure 23. High power electron micrograph of the intestinal mucosa of transgenic potatoes-fed group revealing a mucous gland with homogeneous mucin granules (g). Bar = 0.5 μm

ultrastructure of the intestinal epithelium in both groups of mice fed on δ-endotoxin-treated potatoes and transgenic potatoes. However, in the group of mice fed on the δ-endotoxin-treated potatoes, the Paneth cells of the crypts of Lieberkühn were highly activated and contained a large number of secretory granules. These cells are believed to have an important role in the activation of phagocytes and controlling the bacterial flora of the gut (Ariza *et al.*, 1996; Fawcett, 1997). They contain elevated levels of lysozyme in their large eosinophilic secretory granules, an enzyme capable of digesting bacterial cells walls, and antibacterial peptides called cryptdins (Junqueira *et al.*, 1998). Ouellette (1997) revealed that Paneth cell secretory products seem to contribute both to innate immunity of the crypt lumen and to defining the apical environment of neighboring cells. Wada *et al.* (1993) revealed that the incidence of Paneth cells increases in adenomas and adenocarcinoma, as well as in several other diseased digestive tracts. The antimicrobial polypeptides of the Paneth cell secretory products kill a wide range of organisms, including bacteria, fungi, viruses and tumor cells (Aley *et al.*, 1995).

In conclusion, the present investigation revealed mild changes in the microscopic structure of the different cellular compartments of the ileum of a group of mice fed on transgenic potatoes as compared with another group of mice fed on the δ-endotoxin-treated potatoes, despite the presence of the same type of toxin of *Bacillus thuringiensis* var. *kurstaki* in the transgenic potatoes as a result of gene expression. The appearance of several multinucleated and hypertrophied enterocytes, as well as several associated cytoplasmic fragments with highly recognized annulate lamellae may suggest the possible participation of feeding on the δ-endotoxin-treated potatoes in the hyperplastic development in the mice ileum. Although transgenic crop plants used in food and feed production carry different beneficial transgenes, mostly for resistance to pests, herbicides and diseases (Ondrej and Drobnik, 1997), before releasing for marketing thorough tests and all possible consequences of these new types of heredity and new genetic structures must be evaluated to avoid any potential risks,

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Article

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Intestinal and Peripheral Immune Response to MON810 Maize Ingestion in Weaning and Old Mice

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This study evaluated the gut and peripheral immune response to genetically modified (GM) maize in mice in vulnerable conditions. Weaning and old mice were fed a diet containing MON810 or its parental control maize or a pellet diet containing a GM-free maize for 30 and 90 days. The immunophenotype of intestinal intraepithelial, spleen, and blood lymphocytes of control maize fed mice was similar to that of pellet fed mice. As compared to control maize, MON810 maize induced alterations in the percentage of T and B cells and of $CD4^+$, $CD8^+$, $\gamma\delta$T, and $\alpha\beta$T subpopulations of weaning and old mice fed for 30 or 90 days, respectively, at the gut and peripheral sites. An increase of serum IL-6, IL-13, IL-12p70, and MIP-1β after MON810 feeding was also found. These results suggest the importance of the gut and peripheral immune response to GM crop ingestion as well as the age of the consumer in the GMO safety evaluation.

KEYWORDS: MON810; transgenic maize; mice; intestinal immune response; lymphocytes subpopulations

INTRODUCTION

Interest in genetically modified (GM) crops is continuously increasing due to the possibility of higher agronomic productivity and more nutritious food without the use of pesticides (*1, 2*). The safety issues of GM food are crucial for their acceptance into the market. Although several studies have been conducted to evaluate the safety of GM crops, there is still a debate on the risk of GM consumption and a demand for additional evidence of GM food safety (*3, 4*).

Many trials with animals fed different GM foods such as maize, potatoes, rice, soybeans, and tomatoes have been conducted, and parameters such as body weight, food consumption, organ weight, blood chemistry, and histopathology have been measured. The majority of these experiments did not indicate abnormalities in such parameters (*5, 6*). However, consumption of transgenic pea-α-amylase inhibitor predisposed mice to $CD4^+$ Th2-type inflammation and elicited immunoreactivity to concurrently consumed heterogeneous food antigens (*7*).

The transgenic MON810 maize was produced by insertion of a DNA sequence that encodes a bioactive form of *Bacillus thuringiensis* (Bt) Cry1Ab protein, which is toxic to the corn borer. Protection against corn borer damage may improve yields without the need for chemical insecticide use and reduces the risk of toxigenic fungus infection such as *Fusarium* species (*8, 9*). The safety of MON810 has been evaluated by previous studies that reported no toxicologically significant differences in clinical and neurobehavioral signs, ophthalmology, clinical pathology, organ weights, and gross and microscopic pathology between transgenic and commercial maize fed animals (*5, 6*). The Cry1Ab protein has been also assayed for possible allergenicity. Some authors found that sensitive subjects did not react differently to GM and non-GM samples by skin prick test and IgE immunoreactivity (*10*). However, other authors have reported an increased anti-Bt IgG and IgE response in farm or greenhouse workers (*11, 12*). In addition, a recent study revealed a significant anti-Bt IgG1 response in rats fed a transgenic Bt rice spiked with purified Bt toxin and a tendency to a dose-related response for Bt-specific IgA (*13*).

Until now, assessment of GMO immune adverse effects was based on the potential allergenic evaluation of the pure recombinant proteins, and only a recent study has considered the potential immunotoxicological effects of whole GMO given to rats for different periods (*13*). In addition, no studies have considered the intestinal immune response for such a purpose. However, the intestine interacts continuously with food-derived antigens, allergens, pathogens, and other noxious agents, and the gut immune system, which is the largest lymphoid tissue of the body, is crucial for mounting a correct immune response while maintaining a quiescent status toward innocuous antigens.

In the present study we have evaluated the intestinal and peripheral immune response to long-term MON810 maize consumption, as compared to its parental control and a commercial nontransgenic maize in mice. As gut immune cells, we have considered the intraepithelial lymphocytes (IELs) that form a highly specialized lymphoid compartment and that are the first cells to encounter luminal antigens. These lymphocytes are considered to play an important role in the regulation of immune responses (*14*). We have used both weaning and old mice,

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because they are more susceptible to immunological insults than adult nonaged animals and their immune response may be less efficient.

MATERIALS AND METHODS

Test Materials. Planted seeds derived from MON810 and its parental control maize (PR33P67 and PR33P66 varieties, respectively) were grown simultaneously in neighboring fields in Landriano, Italy (Azienda Agraria Didattico Sperimentale Angelo Menozzi, Milano, Italy). The PR33P67 and PR33P66 seeds were provided by Seeds Emporda (Girona, Spain).

The presence of the Cry1Ab gene in the transgenic maize flour and its absence in the parental control maize flour were checked by MON810 event-specific PCR reactions. DNA was extracted using a CTAB-based protocol (*15*), and real-time PCR was performed using primers, TaqMan probes, and PCR conditions previously described by Kuribara et al. (*16*). The presence of GMO in the control flour was found to be 0.29 ± 0.09%. The results showed the expected DNA band corresponding to the Cry1Ab insertion in the MON810 and not control maize (not shown).

The presence of the protein Cry1Ab in the GM maize was checked and confirmed by ELISA kit (Agdia/Biofords, Evry Cedex, France), according to the manufacturer's instructions.

The presence of mycotoxins aflatoxins B1, B2 G1, and G2, fumonisin B1 (FB1), deoxynivalenol (DON), ochratoxin, and zeralenon was analyzed in the MON810 and control maize by HPLC (Miraglia et al., personal publication). The values were below the maximum allowable concentration, with the exception of FB1, being 1350 and 2450 μg/kg in the transgenic and control maize, respectively (maximum allowable concentration = 2000 μg/kg), and DON, being 1300 and 650 μg/kg in the transgenic and control maize, respectively (maximum allowable concentration = 750 μg/kg).

The micro- and macronutrients compositions of MON810 and its parental maize are reported in a previous study (*17*).

The purified Cry1Ab protein was provided by M. P. Carey (Department of Biochemistry, Case Western Reserve University, Cleveland, OH).

Experimental Diets. The diets were formulated according to the AIN-93G standard diets (*18*) and contained 50% MON810 or its parental control maize flour. A standard pellet diet (Mucedola, Milano, Italy), containing about 50% of a commercial nontransgenic maize, was also used. The absence of Cry1Ab in the pellet diet was confirmed by PCR assay, as described above.

Animals. Male Balb/c mice were used in all of the experiments. Mice at weaning (21 days of age) were obtained from Charles River Laboratories (Como, Italy), whereas old mice (18−19 months of age) were kindly provided by E. Mocchegiani (IRCA, Ancona, Italy). Mice were kept at 23 °C with a 12 h light−dark cycle. Food intake and body weight were recorded every other day. The weaning mice were fed with the different experimental diets for 30 and 90 days, whereas the old mice received the diets for 90 days. The weaning mice fed for 30 days were younger (51 days old) than the 90 day fed mice (111 days old), with different degrees of immune system maturation. Mice had free access to food and water. At the end of the experimental periods, animals were anesthetized with pentobarbital injection (10 mg), blood was drawn via cardiac puncture, and the spleen and small intestine were excised and placed in cold PBS. Animal studies were performed under conditions approved by the National Health Ministry (Department of Food, Nutrition and Public Animal Health).

Lymphocytes Preparation. IELs were isolated from the small intestine according to the method of Corazza et al. (*19*). Briefly, the intestine was washed twice with cold PBS, longitudinally opened, and cut into small size pieces after removal of Peyer patches. Intestinal pieces were washed in Hank's balanced salt solution Ca^{2+} and Mg^{2+} free (HBSS-CMF) and stirred twice for 45 min at 37 °C in HBSS-CMF added with 10% fetal calf serum (FCS, Euroclone, Milano), 1 × 10^5 units/L penicillin, 100 mg/L streptomycin, 5 mM Hepes (pH 7.4), 1 mM EDTA, and 1 mM dithiothreitol. The eluted cells were passed through 100 and 40 μm nylon cell strainers (Becton Dickinson, BD-Falcon, Milano, Italy) and centrifuged at 650*g*. Lymphocytes were isolated by discontinuous 44/67% Percoll (Percoll, GE Healthcare, Milano, Italy) gradient. Spleens were smashed with a 1 mL plastic syringe piston. The released lymphocytes were washed with PBS, separated on Ficoll gradient (Ficoll plaque-plus, GE Healthcare), and resuspended in PBS.

Antibodies for Flow Cytometry. Each antibody was titrated to determine the optimal concentration for maximal staining. The following antibodies were used: FITC anti-CD3 (clone 17.12), PE anti-CD19 (clone 1D3), PerCP anti-CD45 (clone 30-F11), PE anti-CD4 (clone GK1.5), PE-Cy5 anti-CD8 (clone 53−67), PE anti-TCR$\gamma\delta$, (clone GL3), PE-Cy5 anti-TCR-$\alpha\beta$ (clone H57-597), anti-CD16/CD32 (clone 2.4G2). All antibodies were purchased from BD-Pharmingen.

Flow Cytometry. IELs and spleen lymphocytes (1 × 10^6 cells) were preincubated for 20 min with anti-CD16/CD32 to block Fc receptors and avoid nonspecific binding. Cells were then washed and labeled with an appropriate mixture of antibodies or isotype matched controls for 30 min, centrifuged at 650*g*, and resuspended in 0.5 mL of FacsFlow. Blood lymphocytes were analyzed according to the "lyse no wash" protocol from BD. Briefly, 0.1 mL of blood was incubated with an appropriate mixture of antibodies for 30 min and then incubated with erythrocyte lysing solution (155 mM NH_4Cl, 10 mM $KHCO_3$, and 1 mM EDTA) on ice until complete lysis. After centrifugation at 650*g*, the pellet was washed and resuspended in 0.5 mL of FacsFlow. Flow cytometry analysis was performed using a FACSCalibur flow cytometer (BD Biosciences). To exclude dead/dying cells and therefore nonspecific antibody-binding cells, lymphocytes were gated according to forward and side scatter. The percentage of T and B lymphocytes was calculated on leukocyte gate ($CD45^+$), whereas the $CD4^+$, $CD8^+$, $\alpha\beta$T, and $\gamma\delta$T cell subsets were calculated on $CD3^+$ gate. At least 10000 events were acquired and analyzed. Data were analyzed using CellQuest software (BD Biosciences).

Proliferative Assay. The splenic lymphocytes were centrifuged at 250*g* for 5 min and resuspended in RPMI-1640 medium supplemented with 10% FCS, 1 × 10^5 units/L penicillin, 100 mg/L streptomycin, 4 mM glutamine, 1% nonessential amino acids, and 50 mM 2-mercaptoethanol (Sigma, Milano, Italy). Cells were cultured at 3 × 10^5/well in 96-well flat-bottom plates (Corning, Roma, Italy) at 37 °C in a humidified atmosphere with 5% CO_2. Cells were stimulated with 2.5 mg/L of concanavalin A (ConA; Sigma) for 72 h or with pure Cry1Ab (5 mg/L) for 120 h and labeled with 5 mCi/L of ^{3}H-thymidine (6.7 Ci/mmol; NEN, Zaventem, Belgium) for the last 18 h of incubation. After harvesting, radioactivity was counted in a scintillator counter (Microbeta Trilux, Perkin-Elmer, Milano, Italy).

Cytokine Analysis. The levels of serum cytokines were analyzed using a mouse CBA Soluble Flex Set system (BD Biosciences) for interleukin (IL)-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IFN (interferon)-γ, TNF (tumor necrosis factor)-α, MCP-1 (monocyte chemoattractant protein-1), and MIP-1β (macrophage inflammatory protein-1β) detection, according to the manufacturer's specifications. Briefly, multiplexed antibody-conjugated beads were incubated with serum samples or serial dilutions of cytokine standards for 1 h. After PE detection reagent addition, samples were incubated for an additional 1 h, washed, and analyzed by FACScalibur. Results were analyzed using the FCAP1.1 software (BD Biosciences).

Statistical Analysis. The significance of the differences has been tested using the parametric analysis of variance (ANOVA). The SAS statistical package (version 6.12) was used to perform statistical analyses. Differences were considered to be statistically significant when the P value was below 0.05.

RESULTS

Effect of Feeding with MON810 or Its Parental Control Maize on Body Weight and Food Consumption. There were no differences in the mean body weight between mice fed MON810 or its parental control maize for either 30 or 90 days, independent of the age of the animals. No difference was found in the food consumption of weaning and elderly mice fed the MON810 or control maize (Table 1).

Table 1. Body Weight and Food Intake of Weaning and Old Mice Fed MON810 (GM) or Parental Control (C) Maize for 30 or 90 Days[a]

treatment	body wt (g) initial	body wt (g) final	food intake (g/day)
weaning, 30 days			
C	11.3 ± 1.35	22.7 ± 1.35	3.8 ± 0.28
GM	11.2 ± 1.59	23.3 ± 0.90	4.0 ± 0.41
weaning, 90 days			
C	10.3 ± 1.15	30.7 ± 2.60	4.0 ± 0.28
GM	10.3 ± 1.67	29.8 ± 4.22	3.9 ± 0.47
old, 90 days			
C	33.7 ± 2.14	34.8 ± 3.44	4.1 ± 0.31
GM	32.7 ± 3.00	34.4 ± 3.43	4.1 ± 0.38

[a] Data are the means ± SD of at least 15 animals for each group.

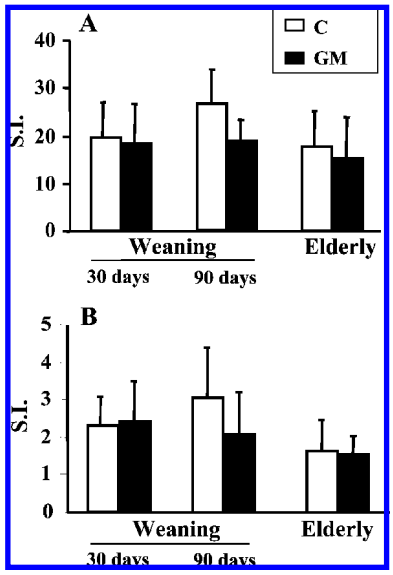

Figure 1. Proliferation of spleen lymphocytes from weaning and old mice fed MON810 (GM) or its parental control maize (C) for 30 or 90 days and stimulated in vitro with ConA (**A**) or Cry1Ab (**B**). The proliferative response was measured as ^{3}H-thymidine incorporation and is expressed as stimulation index (SI, ratio of cpm of stimulated/cpm of unstimulated lymphocytes). Data are the means ± SD of at least 10 animals for each group.

Proliferative Response. To verify whether the lymphocytes maintained the ability to proliferate in response to aspecific or specific stimulus, we measured the proliferation of spleen lymphocytes of weaning and elderly mice fed the MON810 or parental control maize after in vitro stimulation with the polyclonal mitogen ConA or with the purified Cry1Ab. No statistically significant differences were found in the proliferative response to ConA or Cry1Ab in any group of animals (**Figure 1**). However, the stimulation index was low after Cry1Ab stimulation, suggesting a low immunogenicity of Cry1Ab.

Effect of the Transgenic and Nontransgenic Maize Consumption on Lymphocyte Populations. To assess whether the MON810 maize consumption could have immunological consequences, we performed the phenotypic analysis of lymphocytes isolated from the intestinal and peripheral sites of mice fed MON810 or its parental control maize. To exclude any other influence than that caused by the Cry1Ab coding sequence, we also analyzed the lymphocyte subsets of mice fed a standard pellet diet containing a commercial non-GM maize. The immunophenotype of intestinal intraepithelial, spleen, and blood lymphocytes of mice fed the control maize was similar to that of pellet fed mice (data not shown). No difference in the total

Table 2. Total Number of CD45$^+$ Cells from Small Intestine, Spleen, and Blood of C and GM Weaning and Old Mice ($\times 10^6$)[a]

treatment	intestine ($\times 10^6$)	spleen ($\times 10^6$)	blood ($\times 10^6$/mL)
weaning, 30 days			
C	4.4 ± 0.8	27.9 ± 6.1	6.8 ± 1.3
GM	4.1 ± 1.3	24.2 ± 5.8	6.6 ± 2.1
weaning, 90 days			
C	5.2 ± 0.6	28.1 ± 8.4	7.1 ± 1.8
GM	4.9 ± 0.5	29.1 ± 4.9	7.4 ± 1.1
old, 90 days			
C	5.7 ± 0.8	39.2 ± 8.2	7.4 ± 0.9
GM	4.4 ± 1.1	31.7 ± 6.9	7.1 ± 1.4

[a] Data are the means ± SD from at least 10 mice.

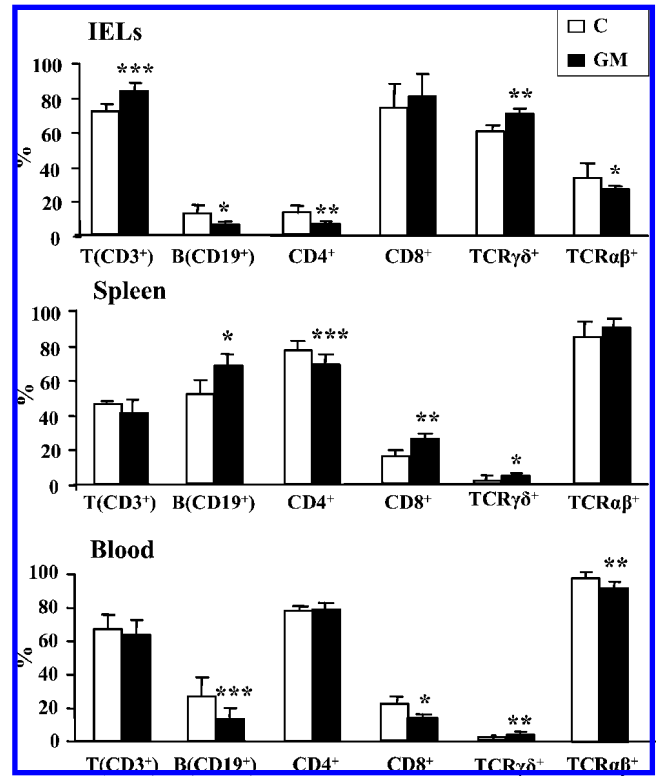

Figure 2. Effect of feeding weaning mice with MON810 (GM) or its parental control maize (C) for 30 days on percentage of lymphocyte populations. The various cell populations of intestinal intraepithelial lymphocytes (IELs) and of spleen and blood lymphocytes were analyzed by flow cytometry. Data represent means ± SD from at least 10 mice. ∗, $P < 0.05$; ∗∗, $P < 0.01$; ∗∗∗, $P < 0.001$, as compared to C.

number of CD45$^+$ cells of the small intestine, spleen, and blood between mice fed MON810 or its parental control maize was found (**Table 2**). Several changes were induced by MON810 maize in the various sites depending on the age of the animals. Indeed, in the weaning mice fed the MON810 maize for 30 days, the amount of T cells was higher in the IELs, whereas the B cells were lower in the IELs and blood and higher in the spleen (**Figure 2**). In addition, the CD4$^+$ subpopulation decreased in the IELs and spleen, whereas the CD8$^+$ subset was higher in the spleen but lower in the blood. The TCR$\gamma\delta^+$ subset was higher in the IELs, spleen, and blood, whereas the TCR$\alpha\beta^+$ subset was lower in the IELs and blood. After 90 days of MON810 maize feeding of weaning mice, only alterations in the percentage of B cells were found, being higher in the IELs and blood (**Figure 3**). In the old mice after 90 days of MON810 maize consumption, the percentage of B cells was lower in the IELs and blood and the CD4$^+$ subset was lower in the IELs

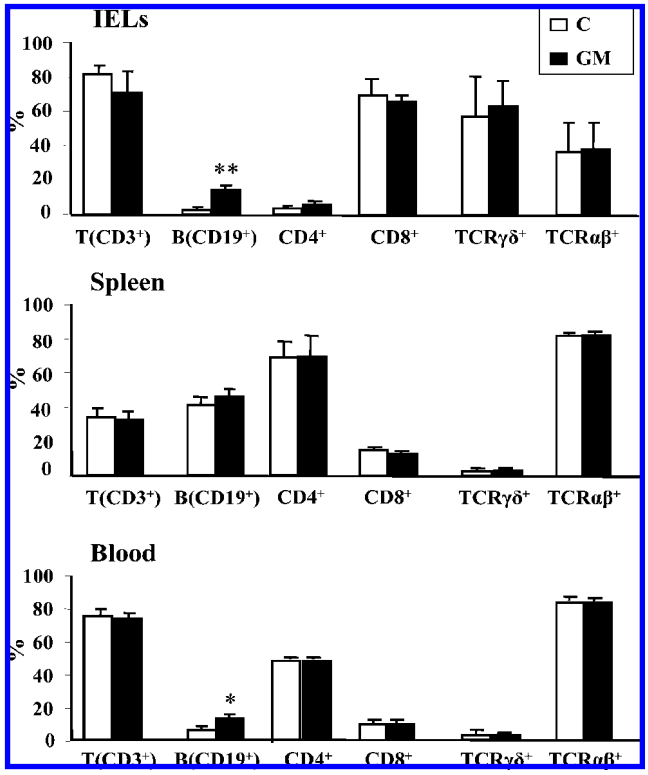

Figure 3. Effect of feeding weaning mice with MON810 (GM) or its parental control maize (C) for 90 days on percentage of lymphocyte populations. The various cell populations of intestinal intraepithelial lymphocytes (IELs) and of spleen and blood lymphocytes were analyzed by flow cytometry. Data represent means ± SD from at least 10 mice. ∗, $P < 0.01$; ∗∗, $P < 0.001$, as compared to C.

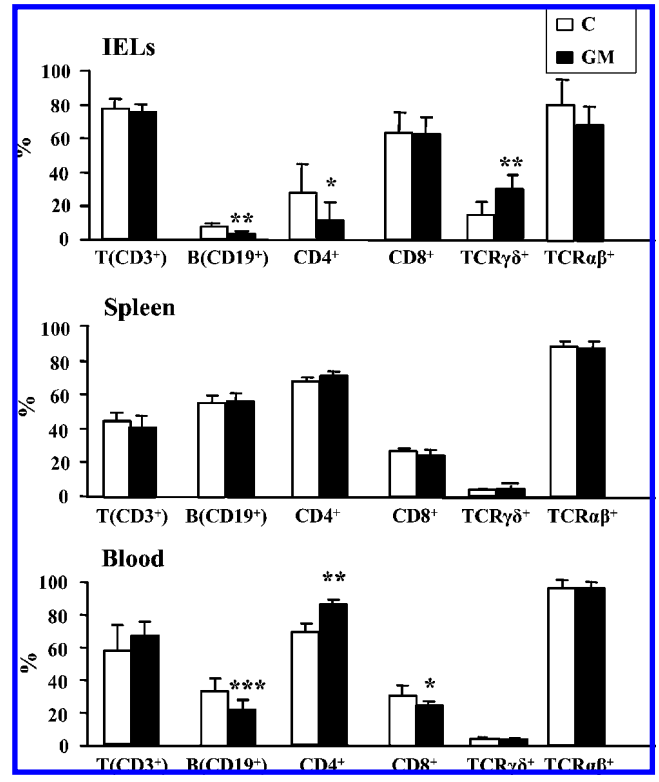

Figure 4. Effect of feeding old mice with MON810 (GM) or its parental control maize (C) for 90 days on percentage of lymphocyte populations. The various cell populations of intestinal intraepithelial lymphocytes (IELs) and of spleen and blood lymphocytes were analyzed by flow cytometry. Data represent means ± SD from at least 10 mice. ∗, $P < 0.05$; ∗∗, $P < 0.01$; ∗∗∗, $P < 0.001$, as compared to C.

and higher in the blood, whereas the $CD8^+$ subset was lower in the blood and the $TCR\gamma\delta^+$ subset was higher in the IELs (**Figure 4**).

Cytokine Profiling. To test whether the MON810 maize consumption induced changes in cytokine pattern, we have evaluated several cytokines in serum of weaning and old mice fed with MON810 or control maize for 30 or 90 days. The results showed an increase in IL-6, IL-13, IL-12p70, and MIP-1β in weaning mice fed MON810 for 30 days, an increase of MIP-1β in weaning mice fed MON810 for 90 days and in old mice, and a small but not significant increase in IL-12p70 in old mice (**Table 3**).

DISCUSSION

In this study we evaluated the immunomodulatory effects of whole transgenic MON810 maize consumption as compared to its parental control and a commercial maize, by considering the gut and peripheral immune response of mice in vulnerable conditions. We report that the MON810 maize used in this study, when given to both weaning and old mice for 30 and 90 days, induced several changes to the immunophenotype of the gut, spleen, and circulating lymphocytes and to the level of serum cytokines.

The MON810 and its parental control maize given to the animals were grown simultaneously in neighboring fields, using the same agricultural techniques and had therefore the same external climatic conditions, which eliminates or reduces environmental variables. In addition, the compositional analysis indicated that both the transgenic and nontransgenic maize had similar nutritional composition, and thus the diets given to the animals were similarly balanced, excluding that the observed effects were caused by improper nutrition. The amount of DON was higher in the transgenic than control parental maize, whereas the amount of FB1 in the control maize was almost double that of the transgenic maize. These mycotoxins are frequent contaminants of maize and may exert immunotoxic activity, depending on dose, exposure, and timing of administration (*20*, *21*). Nevertheless, in agreement with previous studies (*22*), the increases of DON and FB1 were modest, their levels being slightly higher than the maximum allowable concentration and much lower than those known to affect the immune response (*23*−*25*). In addition, the immune markers of the animals fed control maize did not differ from those of animals fed the commercial nontransgenic maize. Thus, all of these data indicate that the observed immunophenotype changes were likely due to the insertion of the Cry1Ab coding sequence.

Several and different perturbations were observed in lymphocyte subsets after MON810 maize consumption, depending on the age of the animals. The most affected were the weaning mice fed for 30 days the transgenic maize, showing several alterations in immunophenotype of IELs, spleen, and blood lymphocytes. Only an increase of B cells was present after MON810 maize consumption in the weaning mice fed for 90 days, which were 2 months older than the weaning mice fed for 30 days. Also, in the old mice the consumption of MON810 maize induced several alterations in the IELs and blood, which resembled those of the weaning mice fed the transgenic maize for 30 days. These data suggest that age was an important factor in the immune response to MON810 maize. This fact is not surprising, considering that the immune system during weaning and aging can less efficiently or inappropriately respond to external stimuli than during adult age. The weaning represents

Table 3. Serum Cytokine Levels of Weaning and Old Mice Fed MON810 (GM) or Parental Control (C) Maize for 30 or 90 Days[a]

	pg/mL										
treatment	IL-4	IL-5	IL-6	IL-10	IL-13	IL-12p70	IL-21	TNF-α	IFN-γ	MIP-1β	MCP1
weaning, 30 days											
C	2.6 (0.3)	2.9 (0.3)	4.3 (1.7)	9.1 (2.3)	2.9 (1.2)	7.0 (0.8)	9.3 (0.7)	14.3 (2.3)	2.1 (0.2)	24.9 (5.1)	39.4 (6.7)
GM	2.8 (0.5)	3.3 (0.5)	20.6 (8.1)*	12.0 (3.9)	6.5 (0.8)*	9.7 (2.9)*	8.1 (3.0)	17.9 (6.6)	2.2 (0.4)	33.0 (6.2)*	60.5 (29.5)
weaning, 90 days											
C	2.6 (0.5)	3.2 (0.5)	3.8 (0.6)	10.2 (3.2)	6.9 (2.7)	8.4 (2.2)	8.6 (0.5)	15.6 (5.2)	2.3 (0.5)	23.3 (3.2)	42.5 (13.4)
GM	2.7 (0.3)	2.9 (0.5)	6.7 (4.7)	9.1 (2.0)	5.6 (1.9)	9.2 (4.0)	10.5 (7.4)	16.8 (2.6)	2.3 (0.6)	32.2 (6.7)*	38.3 (3.2)
old, 90 days											
C	3.2 (0.5)	3.3 (0.6)	5.7 (1.1)	13.1 (1.9)	6.5 (4.1)	10.7 (2.8)	9.4 (3.5)	17.6 (4.1)	2.4 (0.5)	27.0 (5.3)	49.4 (14.5)
GM	2.6 (0.9)	3.4 (0.8)	5.3 (2.8)	11.6 (4.4)	6.3 (1.1)	12.1 (3.1)	7.9 (2.2)	20.6 (5.7)	2.2 (0.7)	39.7 (13.4)*	41.0 (15.9)

[a] Data are the means ± SD (in parentheses). For each column, $P < 0.05$ as compared to C.

a critical point in the development of a balanced immune response to external antigens, because a maximum exposure to novel food antigens together with removal of milk maternal protective factors occurs (*26*−*28*). Nutrition at weaning may also provide new factors that influence intestinal flora, which in turn will affect antigen exposure, immune maturation, and immune responses (*29*−*31*). Problems may arise when the immune system develops and functions inappropriately, resulting in inefficacy to develop tolerance toward harmless food proteins with consequent immunologic disorders (*27*, *32*). In the case of weaning mice fed for 90 days, the low responsiveness to MON810 maize can be due to an acquired ability to tolerate the transgenic food during the longer treatment. With regard to aging, age-associated dysregulations of the immune system are well documented (*33*), and alterations in antigen-specific antibody responses, impairment of oral tolerance, and reduction of natural killer cells are frequently observed (*34*, *35*). In addition, as for weaning, changes in microflora composition occur during aging in a way that may impair the correct immune response (*36*, *37*). In conclusion, our results suggest that age is an important factor to be taken into account in the evaluation of transgenic food safety.

One of the more recurrent alterations in lymphocyte phenotypes observed in this study was an increase in the TCR$\gamma\delta^+$ population. A high percentage of these lymphocytes are localized in the gut and in the mouse, a substantial proportion of $\gamma\delta$T cells resides in the IELs (*38*). $\gamma\delta$T cells seem to be important regulatory elements of the immune system, being capable of modulating inflammatory response associated with infectious agents and autoantigens (*39*−*41*). Higher numbers of $\gamma\delta$T cells have been observed in humans with asthma (*42*), in IELs of children with untreated food allergy (*43*), and in the duodenum of children with juvenile idiopathic arthritis or connective tissue disease with gastrointestinal symptoms (*44*). In addition, murine $\gamma\delta$T cells have been shown to abrogate the oral tolerance (*45*). However, an inhibition of late allergic airway responses and eosinophilia by $\gamma\delta$T cells has also been found (*46*). Besides the exact function of $\gamma\delta$T cells, the significance of the increase of this subpopulation observed in the present study deserves further evaluation. This is certainly true also for the other phenotypic lymphocyte alterations, the meaning of which remains to be defined. For example, the decrease of B cells does not necessarily mean a reduction of their secreted antibodies amount, and it would be interesting to evaluate the impact of MON810 maize on the different classes of antibodies. In this regard, studies are currently evaluating the amount of different antibodies in serum of mice used in the present study, and preliminary results indicate an increase of total IgG and IgE in both weaning an old mice fed MON810 maize as compared to its parental control maize (Ortolani et al., personal publication). A previous study reported no allergenicity of MON810 maize as evaluated by skin prick test in sensitive subjects suffering for asthma-rhinitis or by IgE antibodies secretion against pure Cry1Ab protein in individuals with food allergy (*10*). However, these tests were not performed in vulnerable subjects such as children and elderly people. On the other hand, an anti-Cry1Ab-specific IgG2 response in rats fed transgenic rice expressing Cry1Ab protein for 90 days and increased antigen-specific IgG1 in rats fed for 28 days the same rice but spiked with Bt toxin have been found (*13*). In addition, a study conducted in farm workers exposed to Bt pesticides indicated elevated Bt-specific IgE and IgG antibodies in more high- than low-exposure workers, associated with positive skin prick tests to Bt spore (*11*). Similarly, greenhouse workers exposed to Bt pesticides reported an increase of Bt-specific IgE (*12*).

Alterations of the immunophenotypes induced by the transgenic maize were associated with increased levels in some of the considered cytokines, especially in the weaning mice fed for 30 days the MON810 maize. These cytokines (IL-6, IL-13, IL12p70, MIP-1β) are involved in allergic and inflammatory responses (*47*−*49*), and although they were not strongly elevated by MON810 maize consumption, their increase is a further indicator of immune perturbations induced by MON810 maize.

The recent results obtained by some authors may offer a rationale for the alterations found in the present study, beyond the presence of Cry1Ab protein. Indeed, they have analyzed the seeds of MON810 and its parental control maize utilized in the present study by differential proteomic analysis to evaluate possible unintended side effects. They have found that 43 proteins were up- or down-regulated in transgenic as compared to control seeds, likely as a result of the Cry1Ab gene insertion (*50*). Interestingly, among them a newly expressed 50 kDa γ-zein, a well-known allergenic protein (*51*), was detected. Post-translational modifications in GM crops were also observed in a previous study demonstrating that the transgenic expression of bean α-amylase inhibitor (αA1) in peas led to the synthesis of a modified form of the protein that showed altered antigenic properties (*7*). In addition, consumption of this protein by mice predisposed αA1-specific CD4$^+$Th2-type inflammation.

In conclusion, the results obtained indicate that the consumption of MON810 maize used in the present study induced alterations in intestinal and peripheral immune response of weaning and old mice. Although the significance of these data remains to be clarified to establish whether these alterations reflect significant immune dysfunctions, these results suggest the importance of considering the gut and peripheral immune response to the whole GM crop, as well as the age, in the GMO safety evaluation.

ABBREVIATIONS USED

GM, genetically modified; IELs, intraepithelial lymphocytes, MON810 maize, transgenic maize expressing Cry1Ab protein.

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Safety Testing and Regulation of Genetically Engineered Foods

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Abbreviations: BRAD: US EPA Biopesticides Registration Action Document, EA: Environmental Assessment, EC: European Commission, EPA: US Environmental Protection Agency, FAO: U.N. Food and Agriculture Organization, FDA: US Food and Drug Administration, FFDCA: Federal Food, Drug and Cosmetic Act, FIFRA: Federal Insecticide, Fungicide and Rodenticide Act, GE: genetically engineered, MRID: Master Record Identification Number, OSTP: Office of Science and Technology Policy, PIP: Plant-Incorporated Protectants, SAP: EPA Scientific Advisory Panel, USDA: US Department of Agriculture, WHO: World Health Organization

Introduction

The use of recombinant DNA techniques to engineer food crops with novel traits has aroused tremendous interest and concern throughout the world. Both the public and the scientific community are deeply divided on a host of issues raised by genetically engineered (GE) crops. Do they pose human health or environmental risks? Are they adequately regulated? Should foods containing them be labeled? Should society allow them to be patented? Are they relevant to the developing world? Science alone cannot and will not decide the many disputes that have arisen between and within nations over GE foods. As with the introduction of any powerful new technology, economic, cultural and ethical factors will also come into play. But science can help ground the debate, particularly in the contentious area of regulation.

A thorough understanding of how GE foods are currently regulated is essential because claims regarding the safety of these crops are based largely on assessments by government regulators, which in turn are founded mostly on unpublished studies conducted by the crop developer. Published, peer-reviewed studies, particularly in the area of potential human health impacts, are rare. For instance, the EPA's human health assessment of *Bt* crops cites 22 unpublished corporate studies, with initially only one ancillary literature citation (EPA BRAD, 2001b, pp. IIB32-IIB35).[1] The paucity of peer-reviewed literature is probably due to the reluctance of companies to publish data on their crops on account of intellectual property concerns. This supposition is strengthened by reports concerning independent researchers who have been denied GE crop material by companies, or whose access to such material is

strictly conditioned (Dalton 2002). Thus, the validity of a claim that GE crop X is safe depends almost exclusively upon the quality of both the relevant corporate science and the regulatory approval process. [2]

Here, we will undertake a science-based critique of corporate scientific practices and the US regulatory system with respect to GE foods, with special reference to several commercialized crops and relevant (international) standards. We focus on the US regulatory system because the US has far more GE crops on the market than any other nation, and because American regulatory agencies are so often cited in support of the safety of these foods. We then outline an initial screening regimen for GE foods that, if made mandatory, would in our opinion better protect public health than the current US system.

It should be noted at the outset that this study relies heavily on material largely unknown to the broader scientific community, including several unpublished corporate studies, reports on specific GE crops and their regulation by expert bodies (e.g. committees of the National Academy of Sciences) and documents issued by US regulatory agencies. All of these sources are cited in the reference list, with web addresses where available. The general public may view and copy unpublished studies for non-commercial use at the EPA (see References). The information in this paper that derives from unpublished studies has been made available to the public previously in Freese (2001, 2002, 2003) and in presentations at forums sponsored by the FDA (Food Biotechnology Subcommittee meeting, 8/14/02) and National Academy of Sciences (Committee on Identifying and Assessing Unintended Effects of Genetically Engineered Foods on Human Health, 1/7/03).

Development of US policy

The foundation of the US regulatory system for genetically engineered foods was laid from the mid 1980s to the early 1990s during the Reagan and Bush administrations. The Office of Science and Technology Policy (OSTP 1986) and the Council on Competitiveness (Council, 1991), both White House agencies, decided early on that GE crops and foods would be regulated under existing statutes designed for invasive plants, chemical pesticides and food additives, and that use of recombinant DNA techniques *per se* would not trigger any special regulatory consideration. These policy directives led to the doctrine that later became known as 'substantial equivalence' (for more, see below under Food and Drug Administration). Biotech industry and government officials have testified to the great influence exerted by industry on the formulation of this policy, which was designed to speed transgenic crops to market, while at the same time reassuring consumers that GE foods have passed government review. According to Henry Miller, in charge of biotechnology at the FDA from 1979-1994: "In this area, the US government agencies have done exactly what big agribusiness has asked them to do and told them to do" (as quoted in Eichenwald *et al.*, 2001).

Regulatory purview and performance

Regulation of genetically engineered foods is divided among three federal agencies. The breakdown of regulatory responsibility is as follows:

* The *US Department of Agriculture* oversees GE crop field trials and is responsible for deregulating (i.e. permitting the unregulated cultivation and sale of) GE crops.
* The *Environmental Protection Agency* has jurisdiction over the pesticides in GE pesticidal plants, and has joint responsibility with the Food and Drug Administration for selectable marker genes and proteins used in crop development; and
* The *Food and Drug Administration* conducts voluntary consultations on other aspects of GE foods with those companies that choose to consult with it.

US DEPARTMENT OF AGRICULTURE (USDA)

As of this writing, nearly 40,000 field trials of GE crops have been authorized by the USDA. 84% overall, and 98% in 2002, have taken place under a streamlined "notification" system introduced in 1993 (Caplan 2003). Under this system, the crop developer fills out an application, specifying the plant, the gene transfer method, the transformation vector, the sources of the foreign genetic sequences, and the size and location of the field trial. USDA then notifies the pertinent state department of agriculture and normally issues an "acknowledgement" within 30 days. A somewhat more involved permitting process is reserved for experimental trials involving crops engineered to produce pharmaceuticals or industrial compounds (NAS 2002).

The USDA has established guidelines (performance standards) for GE crop trials (USDA Performance 2001). The Department's chief concern is to minimize gene flow to, and inadvertent mixing with, conventional crops and weeds. However, USDA's recent admission that there have been 115 compliance infractions by GE crop field trial operators raises serious doubts as to the efficacy of its regulation (USDA Compliance 2003). Two contamination episodes involving field trials of biopharmaceutical corn in the fall of 2002 highlight the inadequacy of USDA's oversight in this regard (Ferber 2003). It remains to be seen whether the Department's subsequent strengthening of permit conditions and oversight for pharmaceutical and industrial crops will prevent contamination of food-grade crops (USDA Notice, 2003). The issue of contamination is especially important given the *de facto* zero tolerance standard for such compounds in food and feed. In addition, many of the field trial sites falling under the notification system are never visited by a USDA inspector (NAS, 2002).

USDA also clears GE crops for commercial cultivation through issuance of a "determination of nonregulated status." As of this writing, 60 petitions for nonregulated status have been approved. Though some petitions have been withdrawn, the USDA has not explicitly denied any petitions, though one is listed as "void" (USDA Deregulated, 2003). The Department requires considerably more data for deregulation than for field trials, but deregulation is absolute, completely removing the crop and all its progeny from the USDA's regulatory authority (NAS, 2002). In line with its governing statute, the Plant Pest Act, the USDA's chief criterion for deregulation is the lack of invasive or "weedy" characteristics. The USDA has no authority to evaluate the potential health impacts of the crop, or of conventional crops that become contaminated with experimental traits. And since there is no mandatory review by the FDA (see below), GE crops can theoretically enter the marketplace with no review of potential health impacts.

However, even the adequacy of USDA's evaluation of the weediness potential of a GE crop is open to question. For instance, in 1998 the USDA cleared AgrEvo's [now Bayer CropScience] Liberty Link glufosinate-tolerant rice for commercial cultivation despite its recognition that "the *bar* gene conferring tolerance to glufosinate will introgress into red rice and could result in a glufosinate-tolerant red rice population" (USDA Determination, 1998). The USDA had earlier recognized that red rice is a weed that "causes problems in rice fields because it is carried with cultivated rice and can significantly lower its value by reducing [sic] its processing characteristics" (USDA EA, 1996). Nevertheless, the Department stated that "these hybrid offspring [glufosinate-tolerant red rice] will still be sensitive to other registered herbicides" (USDA Determination, 1998). This lack of concern is surprising in view of the USDA's admission, in the very same deregulation notice, that varieties of rice resistant to two other herbicides (imidazolinone and glyphosate) are under development. If the USDA deregulates the latter two varieties as well, they may help foster the development of doubly- or triply-resistant weedy red rice. Multiple herbicide resistance is not unprecedented. For example, three types of canola, two genetically engineered and one mutated for resistance to a different herbicide each, are planted in western Canada. The emergence of volunteer canola plants resistant to one, two and even three herbicides is considered to be "a

major weed problem" in some parts of Canada, with the potential to become "one of Canada's most serious weed problems..." (RS Canada 2001).

A committee of the National Academy of Sciences recently reviewed the USDA's performance at regulating GE crops. Some of the many deficiencies it found include lack of transparency, too little external scientific and public review of decision-making, poorly trained personnel, and allowing companies to make excessive claims of confidential business information (CBI). In fact, the committee itself complained that it was denied access to information it needed to conduct its review due to inaccessible CBI (NAS, 2002).

ENVIRONMENTAL PROTECTION AGENCY (EPA)

The EPA's primary role is regulation of the plant pesticides in crops such as genetically engineered *Bt* corn, cotton and potatoes[3]. *Bt* crops are engineered to produce an insecticidal protein derived from the bacterium *Bacillus thuringiensis*. In 2003, *Bt* corn varieties comprised 29% of all US corn, while 41% of US cotton contained a *Bt* trait (NASS, 2003). *Bt* potato plantings shrank from a peak of about 50,000 acres in 1998 and 1999 to 5,000 acres in 2000, due primarily to the decision of fast-food giants McDonald's and Burger King to source only non-*Bt* potatoes (EPA BRAD, 2001d, pp. I24-I25; Kilman, 2000).

The EPA is responsible not only for the environmental, but also the potential human health impacts of plant-generated GE pesticides. The EPA registers plant pesticides under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), while it has the power to set maximum allowable levels (tolerances) of plant pesticides in crops under the Federal Food, Drug and Cosmetic Act (FFDCA). The EPA has exempted *Bt* plant pesticides from tolerances in all crops (i.e. allowed unlimited amounts), save for StarLink corn, which was never approved for food use. In line with its ruling statutes, which were formulated for chemicals rather than living organisms, the EPA explicitly disavows authority over any aspects of the GE plant beyond its incorporated pesticide. This includes any potential unintended effects, which are supposedly regulated by the FDA (EPA PIP, 2001).

Unlike the FDA, which has a voluntary consultation process, companies developing GE pesticide plants must consult with the EPA. However, the EPA has failed to establish data requirements specific to plant pesticides (EPA PIP, 2001). In the meantime, the Agency has referred developers of GE pesticide-producing crops to a nearly decade-old guidance (EPA Statement of Policy 1994). This Statement of Policy devotes just 4 short paragraphs to testing for human health effects. The Agency recommends only that companies conduct short-term oral toxicity tests in rodents and *in vitro* digestibility tests on the plant pesticide, without any guidance on or specification of test conditions. One strength of EPA regulation is the Agency's ample use of Scientific Advisory Panels, outside experts called in to advise the EPA on issues where it lacks adequate expertise. However, the EPA frequently does not follow the recommendations of its expert advisers with respect to data requirements for product characterization, evaluation of potential human health impacts and specification of test conditions (see Case study - *Bt* corn below).

The quality of corporate environmental studies, and the EPA's review of them, can also be questionable. For example, feeding studies designed to detect potential effects of GE pesticidal proteins on non-target insects such as honeybees are often too short to give meaningful results, for instance 9 days (see Maggi and Sims 1994, Hilbeck and Meier 2002). However, the EPA often accepts such inadequate studies as substantiating the hypothesis that GE pesticidal proteins are not harmful to insects at the tested doses (EPA BRAD 2001a; Mendelsohn *et al.*, 2003). Hilbeck and Meier (2002) recommend full life-cycle testing to detect sub-lethal and long-term effects.

Finally, the EPA plays a critical role in the introduction of herbicide-tolerant plants by raising or establishing tolerance levels for herbicide residues on crops. For instance, in 1992 Monsanto successfully petitioned the EPA to raise the tolerance for glyphosate residues on soybeans from 6 to 20 ppm (EPA Rule, 1992). This anticipated the introduction, several years later, of glyphosate-tolerant soybeans (Lappe and Bailey, 1998), which are associated with greater usage of glyphosate than conventional soybeans (Benbrook, 2001, 2003). The EPA recently granted a petition from Bayer CropScience, whose glufosinate-tolerant rice had already been deregulated by the USDA, to establish a tolerance for residues of glufosinate on rice (EPA, 2003).

FOOD AND DRUG ADMINISTRATION (FDA)

The US regulatory agency most commonly cited as vouching for the safety of GE foods exercises the least authority in regulating them. Theoretically, transgenic proteins in foods fall under the "food additives" provisions of the Federal Food, Drug and Cosmetic Act (FFDCA). Food additives must undergo extensive pre-market safety testing, including long-term animal studies, unless they are deemed to be "generally recognized as safe" (GRAS). The FDA has left it up to the biotech industry to decide whether or not a transgenic protein is GRAS, and so exempt from testing (FDA Policy, 1992). The FDA has yet to revoke an industry GRAS determination and require food additive testing of any transgenic crop[4].

This blanket GRAS exemption is based on the notion of "substantial equivalence" – the strong, *a priori* presumption that GE crops are largely the same as their conventional counterparts. This assumes not only the safety of the transgenic protein, but also the absence of any potentially harmful, unintended effects of transformation. When this policy was being formulated in the early 1990s, scientists at the FDA raised numerous objections to a working draft of the policy (FDA Memos). For instance, FDA scientists at the Division of Food Chemistry and Technology and the Division of Contaminants Chemistry called for mandatory review, stating that "every transformant should be evaluated before it enters the marketplace" (FDA Memo 1991). Dr. Samuel Shibko, Director of the Division of Toxicological Review and Evaluation, recommended "a limited traditional toxicological study with the edible part of the plant," as well as "limited studies in humans" and *in vitro* genotoxicity tests (FDA Memo, 1992a). The most commonly expressed concern was unintended effects associated with the random nature of transformation techniques. Dr. Louis J. Pribyl's comments are typical: "When the introduction of genes into plant's genome randomly occurs, as is the case with the current technology (but not traditional breeding), it seems apparent that many pleiotropic effects will occur. Many of these effects might not be seen by the breeder because of the more or less similar growing conditions in the limited trials that are performed." Pribyl also raised concerns about "new, powerful regulatory elements being randomly inserted into the genome" that could cause "cryptic pathway activation" that breeders might miss. "This situation is different than that experienced by traditional breeding techniques [sic]" (FDA Memo, 1992b). Administrative superiors at the FDA and the White House apparently did not heed these concerns, resulting in today's voluntary consultation process.

Under voluntary consultation, the GE crop developer is encouraged, but not required, to consult with the FDA. The company submits data summaries of research it has conducted, but not the full studies. That is, the FDA never sees the methodological details, but rather only limited data and the conclusions the company has drawn from its own research. As one might expect with a voluntary process, the FDA does not require the submission of data. And in fact, companies have failed to comply with FDA requests for data beyond that which they submitted initially (Gurian-Sherman, 2003). Without test protocols or other important data, the FDA is unable to identify unintentional mistakes, errors in data interpretation or intentional deception, making it impossible to conduct a thorough and critical review.

The review process outlined above makes it clear that, contrary to popular belief, the FDA has not formally approved a single GE crop as safe for human consumption. Instead, at the end of the consultation, the FDA merely issues a short note summarizing the review process and a letter that conveys the crop developer's assurances that the GE crop is substantially equivalent to its conventional counterpart. The FDA's letter to Monsanto regarding its MON810 *Bt* corn is typical:

> "Based on the safety and nutritional assessment you have conducted, it is our understanding that Monsanto has concluded that corn products derived from this new variety are not materially different in composition, safety, and other relevant parameters from corn currently on the market, and that the genetically modified corn does not raise issues that would require premarket review or approval by FDA. ... as you are aware, it is Monsanto's responsibility to ensure that foods marketed by the firm are safe, wholesome and in compliance with all applicable legal and regulatory requirements" (FDA Letter, 1996).

In its official capacity, the FDA carefully avoids vouching for the safety of GE foods, consistent with its voluntary review process. Clearly, the FDA does not send such letters to drug companies or makers of food additives. In these cases, the agency conducts an exhaustive review of a full set of required studies on the product, then either approves or rejects it on its own authority.

Under the voluntary consultation system, the FDA cannot adequately fulfill its role of reviewing GE foods for the presence of toxins or allergens, alterations in nutritional content, unintended effects of the transformation process, or any other food safety concerns not related to GE pesticidal proteins (which come under EPA's purview). For example, in its consultation with Aventis on the company's GE male-sterile corn, the FDA apparently raised no concerns about Aventis' failure to test for possible expression of the pollen-sterilizing GE toxin barnase (a ribonuclease derived from *Bacillus amyloliquefaciens*) in kernels, leaves or other non-pollen corn tissues (FDA Note, 2000), despite evidence that bacterial barnase causes kidney damage in rats (Ilinskaya and Vamvakas, 1997; for an analysis, see Freese, 2003). Another example of the FDA's inadequate performance is detailed below in the case study of *Bt* corn. This case study is preceded by a summary of what we believe to be the major shortfalls in voluntary corporate testing procedures.

Corporate testing procedures

Though not required to do so by the FDA, GE crop developers do test their novel plants in a variety of ways. Given the weaknesses in the regulatory system described above, the quality and scope of corporate testing become key factors in evaluating claims concerning the safety of GE crops. Three especially troubling issues are detailed below.

Surrogate Proteins

Biotechnology companies rarely test the transgenic protein actually produced in their engineered crops. Instead, for testing purposes they make use of a bacterially generated surrogate protein that may differ in important respects from the plant-produced one. The same genetic construct used to transform the plant is expressed in bacteria (usually *E. coli*), and the surrogate transgenic protein is then extracted from the bacteria. This surrogate protein is then employed for all subsequent testing, such as short-term animal feeding studies and allergenicity assessments. This is, however, a serious mistake in testing paradigms, since plants and bacteria are very likely to produce different proteins even when transformed with the

same gene (for discussion, see Schubert, 2002). Testing a bacterial surrogate should not substitute for testing the plant-expressed proteins for the following reasons:

DNA transfected into both plants and animals is incorporated randomly into chromosomal DNA and in doing so may disrupt the function of the chromosomal gene into which it is incorporated, contributing to the unpredictable nature of GE organisms. In addition, only part of the transfected DNA sequence may be incorporated and expressed, and additional problems arise if a fusion protein is made from both transfected and host DNA. For instance, Monsanto and Novartis developed a glyphosate-tolerant sugar beet line in which only 69% of one of the transgenes was incorporated, resulting in fusion with sugar beet DNA and production of the corresponding novel fusion protein (FDA Note, 1998). Even if precisely the same foreign DNA is expressed in bacteria and plant, the two organisms – which are kingdoms apart in biological terms – process proteins differently. For instance, bacteria are not known to add sugar molecules to proteins, while plants do. Glycosylation patterns influence the immune response to proteins, and glycosylation is considered to be a characteristic of allergenic proteins (SAP MT, 2000, p. 23). Other secondary modifications will certainly occur when proteins are expressed in foreign organisms or different cell types (Schubert, 2002). As a result, animal feeding studies and allergenicity assessments that make use of bacterial surrogate proteins or their derivatives may not reflect the toxicity or allergenicity of the plant-produced transgenic protein to which people are actually exposed.

Biotech companies use surrogate proteins for testing purposes because they find it difficult to extract sufficient quantities of the transgenic proteins from their plants (for *Bt* crops, see: EPA BRAD, 2001b, pp. IIA3-IIA4; for glyphosate-tolerant soybeans, see Harrison *et al.*, 1996). Yet several expert bodies on both sides of the Atlantic have criticized this practice. The Scientific Steering Committee of the European Commission calls for demonstration of "chemical identity (including conformational identity)" between surrogate and plant-produced proteins before accepting the former for testing purposes (EC, 2000). According to a National Academy of Sciences committee that conducted an exhaustive review of *Bt* crops (NAS, 2000): "Tests should preferably be conducted with the protein as produced in the plant." If surrogates are nonetheless used: "The EPA should provide clear, scientifically justifiable criteria for establishing biochemical and functional equivalency when registrants request permission to test non plant-expressed proteins in lieu of plant-expressed proteins." Three years later, the EPA has still failed to do this, even though its scientific advisers have proposed such "test substance equivalence" criteria (SAP MT 2000, p. 14). In fact, the toxicity and allergenicity assessments of the major *Bt* corn and cotton events currently on the market employed surrogate proteins that did not meet these criteria (Freese, 2001).

Immunologic differences between plant-produced and bacterial surrogate proteins could have serious medical consequences. An EPA Scientific Advisory Panel (SAP) with some of the nation's leading allergists was convened to evaluate cases of allergic reactions from consumption of food potentially contaminated with StarLink corn, which produces the Cry9C insecticidal protein. This SAP criticized the FDA for using a bacterial surrogate Cry9C rather than StarLink corn Cry9C in its allergy assay (an ELISA to detect antibodies to Cry9C in sera): "The use of non-equivalent, bacteria-derived coating antigen raises the possibility that IgE directed against plant derived Cry9C may not be detected." For this and other reasons: "The test, as conducted, does not eliminate StarLink Cry9C as a potential cause of allergic symptoms" (SAP StarLink, 2001). In fact, the advisors cautioned that any level of StarLink in food might be harmful: "... the Panel concluded that based on reasonable scientific certainty, there is no identifiable maximum level of Cry9C protein that can be suggested that would not provoke an allergic response and thus would not be harmful to the public" (SAP StarLink 2001).

A protein generated in a foreign host may also exhibit point mutations relative to the native protein that can alter the protein's immunogenicity and allergenicity (Wal, 1998). Yet regulators do not demand full sequencing data. Instead, they usually accept company studies comparing 5-25 amino acids at the N-terminal of surrogate and plant-produced proteins as sufficient for a demonstration of sequence

equivalence. For example, EPA's review of Cry1F corn states: "N-terminal sequencing of 5 aa determined that the microbial and plant expressed protein maintained this sequence intact." Yet five amino acids represent less than 1% of the 605 amino acids in plant-expressed Cry1F (EPA BRAD, 2001c). Given the use of bacterially produced surrogate proteins as the norm for testing, one cannot avoid the conclusion that the plant-produced transgenic proteins we actually eat are virtually untested.

UNINTENDED EFFECTS

The artificial introduction of foreign genetic constructs into plant cells creates numerous opportunities for potentially hazardous, unintended effects. These include the over-production of native allergens or toxins, nutritional deficits, and, as discussed above, the creation of novel fusion proteins with unknown properties. Unintended effects are common in all cases where GE techniques are used. For example, engineering a human gene into human cells significantly increases or decreases the expression levels of 5% of the genes in the cell (see Schubert, 2002 for discussion). Excess lignin production in *Bt* corn (Saxena and Stotzky, 2001), reduced levels of certain phytoestrogens in glyphosate-tolerant soybeans (Lappe *et al.*, 1998) and unpredicted changes in the small molecule metabolism of GE potatoes (Roessner *et al.*, 2001) are three of many examples of unintended effects in GE crops (see also Kuiper *et al.*, 2001, Haslberger, 2003).

As stated above, these issues were recognized by FDA scientists in the early 1990s, but their recommendations to require testing for unintended effects were overruled. As a result, the FDA is usually only given summary data on overall fat, protein and carbohydrate levels, together with measurements of a handful of compounds, such as amino acids and selected nutrients. In contrast, European scientists advocate non-targeted techniques for measuring the levels of hundreds of proteins, metabolites, and mRNAs to increase the chances of detecting unintended effects (Kuiper et al, 2001, Kok and Kuiper, 2003), as we do below.

TEST PROTOCOLS

There are very few established protocols for assessing the potential human health impacts of GE crops. Instead, one finds loose guidelines that in most cases only list certain tests or procedures without specifying how they are to be conducted. Allergenicity test guidelines are an important case in point. Since 1996, various groups have devised so-called "decision trees" that lay out a series of tests (e.g. sequence comparison to known allergens, digestive and heat stability, sera screening, etc.) to assess the potential allergenicity of transgenic crop proteins (e.g. Metcalfe *et al.*, 1996). However, until a 2001 report by an FAO-WHO expert consultation (FAO-WHO, 2001), none of these decision-trees specified test conditions. As a result, biotech companies have been free to devise procedures of their own choosing that often vary markedly from tests conducted by independent researchers (see Case study - *Bt* corn below). Clearly, the identification and standardization of these tests is required to facilitate rigorous review. The FAO-WHO expert consultations and emerging *Codex Alimentarius* standards are a step in the right direction (Haslberger, 2003).

The following case study of *Bt* corn illustrates some of the shortcomings in corporate testing and government regulation outlined above.

Case study – *Bt* corn

Bt corn is planted on over 20 million acres in the US alone, making it the most widely planted GE crop after herbicide-resistant soybeans. Corn is a staple in many African and Latin American societies, sweet corn is popular in the US, and corn derivatives are common in processed foods. *Bt* corn therefore deserves close examination for potential human health impacts.

Bacillus thuringiensis (*Bt*) is a soil microbe that produces a variety of insecticidal endotoxins. Microbial *Bt* insecticides targeting lepidopteran pests contain *Bt* proteins of the Cry1 class, and are widely used in spray form by organic and conventional farmers to control the European corn borer (Hilbeck *et al.*, 2000). One of the major insecticidal proteins in *Bt* sprays is known as Cry1Ab. Modified versions of Cry1Ab are engineered into Monsanto's MON810 and Syngenta's *Bt*11 corn events. Corn hybrids descended from these two events, which were first approved by the EPA in 1996, comprise the majority of *Bt* corn in the fields. While there has been very little independent testing of *Bt* corn and other *Bt* crops for potential human health impacts, a few studies conducted on the related *Bt* sprays raise concerns about the potential allergenicity of *Bt* corn.

Our concerns derive from four sources: 1) Suggestive evidence of allergenicity from human and animal studies as well as allergen-like properties of the *Bt* insecticidal protein Cry1Ab; 2) Unintended consequences of the genetic engineering process; 3) Regulatory failure; and 4) Differences between insecticidal proteins in *Bt* sprays and *Bt* crops.

SUGGESTIVE EVIDENCE OF ALLERGENICITY

Allergic symptoms including allergic rhinitis, angioedema, dermatitis, pruritus, swelling, erythema with conjunctival injection, exacerbations of asthma, angioedema and rash have been reported in farm workers and others exposed to *Bt* spraying operations (Bernstein *et al.*, 1999). Bernstein *et al.* demonstrated that purified Cry protein extracts of *Bt* microbial pesticides containing Cry1Ab and Cry1Ac elicited positive skin tests and IgE antibody responses in two farm workers exposed to these toxins by the inhalational, dermal and possibly oral routes. Positive skin tests and the presence of IgE antibodies in serum are considered indicators of allergenicity. Though Bernstein *et al.* did not observe allergic reactions in these workers, they note that the workers were tested after only 1 to 4 months of exposure, and that "clinical symptoms would not be anticipated unless there was repeated long-term exposure…" In addition, they note that the "healthy worker effect" might have skewed their results – that is, susceptible farm workers might have associated their allergic symptoms with *Bt*, sought other employment to avoid exposure, and hence not been included in their study.

Additional evidence for the allergenicity of *Bt* endotoxins is provided by Vazquez and colleagues in a series of animal studies demonstrating that both Cry1Ac protoxin (inactive precursor of the toxin) and toxin are potent immunogens, eliciting both mucosal and systemic immune responses (Vazquez *et al.*, 1999a, 2000a), and that Cry1Ac protoxin is a systemic and mucosal adjuvant similar in potency to cholera toxin (Vazquez *et al.*, 1999b). They also found that Cry1Ac binds to surface proteins in the mouse small intestine (Vazquez *et al.*, 2000b). It should be noted that Cry1Ac is very similar in structure to the Cry1Ab insecticidal protein in most varieties of *Bt* corn. However, binding tests on Cry1Ab have yielded negative or ambiguous results. No specific binding to GI tract tissues was found in an *in vivo* test with an *E. coli*-generated surrogate Cry1Ab in rats, though some binding, described as "aspecific," was found *in vitro* in caecum and colon tissue of the rhesus monkey (Noteborn *et al.*, 1995).

In an assessment of *Bt* crops, expert advisors to the EPA who reviewed the Bernstein study and one of Vazquez *et al.*'s four studies concluded that: "These two studies suggest that *Bt* proteins could act as antigenic and allergenic sources" (SAP *Bt*, 2000, p. 76). Different approaches were called for to further

characterize the allergenic risk of *Bt* proteins: "Only surveillance and clinical assessment of exposed individuals will confirm the allergenicity of *Bt* products..." (SAP *Bt*, 2000, p.76). Finally, the EPA's experts noted that testing for potential reactions to Cry proteins in *Bt* spray and *Bt* crops could be undertaken now: "The importance of this [Bernstein's] report is that reagents are available that could be used for reliable skin testing and serological evaluation of *Bt* protein exposed individuals." Unfortunately, in 2001 the EPA re-registered *Bt* corn for 7 years without making use of these reagents (EPA BRAD, 2001d, p. I2). The Agency has also discounted other evidence of the potential allergenicity of *Bt* proteins.

This evidence relates to physical characteristics of the *Bt* corn protein (Cry1Ab) that are considered typical features of food allergens by expert groups that have devised decision-tree protocols designed to screen novel transgenic proteins for allergenic potential (e.g. Metcalfe *et al.*, 1996, FAO-WHO, 2001). Three of these characteristics are amino acid sequence homology to a known allergen, digestive stability and heat stability. While none of these features is *predictive* of allergenicity, their presence (especially in combination) is regarded as sufficient evidence to reject the pertinent GE crop, or at least trigger additional testing, depending on the protocol. While the EPA ostensibly "requires" data on these three parameters for all *Bt* crop proteins "to provide a reasonable certainty that no harm will result from the aggregate exposure" to them (EPA BRAD 2001b, p. IIB1), in practice it has simply not collected pertinent studies, accepted substandard ones, or ignored relevant evidence.

For instance, the EPA apparently did not make use of a study by FDA scientist Steven Gendel that demonstrated sequence homology between several Cry proteins and known food allergens. Homology of sequences 6 to 8 amino acids in length are considered potentially significant because allergenic epitopes can be this small (Metcalfe *et al.*, 1996, FAO-WHO, 2001). Gendel found that Cry3A (*Bt* potatoes) and β-lactoglobulin, a milk allergen, shared sequences 7-10 amino acids in length. He also identified sequences of 9-12 amino acids shared by Cry1Ab (*Bt* corn) and vitellogenin, an egg yolk allergen. Gendel concluded that: "…the similarity between Cry1A(b) and vitellogenin might be sufficient to warrant additional evaluation" (Gendel, 1998). The EPA knew about this study because it had been discussed by its scientific advisers (SAP MT 2000). But the Agency re-registered *Bt* corn for 7 years in 2001 without discussing or even citing Gendel's study in its review document, with no corresponding study on file from Syngenta, and only incomplete data from Monsanto (EPA BRAD, 2001b, p. IIB4).

Many food allergens are stable to digestion. It is thought that the longer a protein survives in the gut, the more likely it is to induce the cascade of immune system events leading to allergic sensitization and reaction in susceptible individuals. Most food proteins, both native and transgenic, break down rapidly in the gut due to the action of protein-degrading enzymes and acid. Transgenic proteins (or rather, their bacterial surrogates) are normally tested *in vitro* in acidic solutions containing pepsin. The rate of breakdown is significantly influenced by the amount of pepsin relative to test protein in, and the acidity of, the simulated gastric fluid.

Two digestive stability studies on Cry1Ab, the GE toxin found in *Bt* corn, by Hubert Noteborn established that: 1) After 30-180 minutes in simulated gastric fluid (SGF), 9-21% of Cry1Ab remains undigested; 2) After 2 hours in SGF, Cry1Ab degrades only to fragments of substantial size at the low end of the range considered typical of food allergens (15 kilodaltons); and 3) Cry1Ab is substantially more resistant to digestion than four other transgenic proteins tested, including one other Cry protein, Cry3A. Of the six proteins Noteborn tested, only StarLink corn's Cry9C exhibited greater digestive stability (Noteborn *et al.*, 1995, Noteborn, 1998). In contrast, industry procedures used to measure digestive stability frequently employ highly acidic conditions and a very large excess of pepsin relative to test protein – conditions that favor the most rapid possible digestion (e.g. Ream 1994). Under the authoritative allergenicity testing protocol recommended by international experts at FAO/WHO, digestive stability tests are to be carried out at a higher pH (2.0) and in SGF with a ratio of test protein to pepsin

over three orders of magnitude greater than the conditions used by some (FAO-WHO, 2001). Thus, it's no surprise that protein stability results may vary by a factor of up to 60. These conflicting reports show the need for standardized testing procedures.

Finally, Noteborn also found that Cry1Ab possessed "relatively significant thermostability … comparable to that of the Lys mutant Cry9C protein" found in StarLink corn (Noteborn, 1998). Noteborn found that Cry9C was stable for 120 minutes at 90° C, but gives no further information on Cry1Ab's heat stability. The EPA failed to collect any heat stability study from Monsanto on MON810 (EPA BRAD, 2001b, p. IIB4). For further analysis of the data discussed above, see Freese (2001).

UNINTENDED CONSEQUENCES OF THE GENETIC ENGINEERING PROCESS

Many *Bt* corn hybrids planted on millions of acres in the US are derived from Monsanto's MON810 event, which contains the Cry1Ab insecticidal toxin discussed above. However, an unpublished molecular characterization study on MON810 reveals that the genetic construct broke apart during the transformation process, resulting in several unintended consequences (Levine *et al.*, 1995). The following aberrant transfection events were noted: 1) An undefined portion of the E35S enhanced cauliflower mosaic virus promoter was incorporated into MON810; 2) Only a fragment (about 70%) of the intended full-length cry1Ab protoxin gene was incorporated; 3) Thus, by definition the NOS termination sequence was not integrated; instead, the cry1Ab gene fragment fused with enough DNA to code for 2 amino acids (Levine *et al.*, 1995), DNA that apparently derives from the host plant. These unexpected transfection events create the potential for production of a fusion protein. Yet Western blots apparently did not reveal the predicted expression product of the open reading frame, a 92 kD fusion protein, but rather only a 63 kD "tryptic core" protein. Levine *et al.* speculate that their failure to detect the putative 92 kD fusion protein is "probably due to low expression or rapid degradation to the trypsin-resistant product during the extraction procedure." The authors do not report any formal experiment to test either of these possibilities.

In addition, Lee *et al.* (1995) and Lee and Bailey (1995) report that the safety testing for MON810 and related *Bt* corn lines employed a bacterial surrogate Cry1Ab made in *E. coli*, not the fusion protein apparently produced by MON810. These two studies attempt to demonstrate equivalence between the plant-produced and bacterial surrogate Cry1Ab proteins to justify use of the latter in safety testing, yet the equivalence testing compared only the trypsin-generated cores of the plant and bacterial proteins. Results of testing with this bacterial surrogate clearly may not reflect the toxic and allergenic profile of the putative corn-produced fusion protein. Thus, the properties of the plant-expressed protein remain largely unknown (see Freese, 2001 for a fuller discussion).

Whatever partial *Bt* fusion protein is produced by MON810, it confers insect resistance, the crop developer's chief concern. But regulatory officials should demand more. The EPA, which has jurisdiction over the plant pesticide, merely noted in its review document that MON810 produces a "truncated" Cry1Ab protein (EPA BRAD 2001b, p. IIA6), saying nothing about integration of a gene fragment or generation of a fusion protein. The FDA, which is supposed to review the whole GE plant (even pesticidal plants like MON810) for unintended effects, nutritional deficits, etc., states in its consultation note that MON810 contains 1 complete copy of the cry1Ab gene, a NOS termination sequence, and a "nature-identical" Cry1Ab protein, none of which is correct (FDA Note 1996). Apparently, either Monsanto submitted incomplete summary data to the FDA, or the FDA made serious errors in its consultation note. In either case, it is troubling that the US agency responsible for food safety has fundamentally flawed molecular characterization data on such a widely planted GE crop. In general, we believe that the presence or potential presence of a novel fusion protein in a GE crop should trigger a mandatory review for potential human health or environmental impacts.

Bt corn exhibits another striking unintended effect. *Bt* corn hybrids descended from Monsanto's MON810 and Syngenta's *Bt*11 events have markedly increased levels of lignin in stem tissue (Saxena and Stotzky, 2001). This finding is in accord with anecdotal reports from farmers that *Bt* corn is stiffer and less desirable to farm animals as fodder, for lignin is the woody component of plants and is non-digestible. Lignin is the polymeric product of three aromatic compounds, coniferyl alcohol, p-coumaryl alcohol and sinapyl alcohol, all of which are derived from phenylalanine, an essential aromatic amino acid (Humphreys and Chapple, 2002). Phenylalanine, in turn, is a product of the shikimic acid pathway, which is responsible for generating compounds comprising 35% and more of the dry mass of higher plants (Alibhai and Stallings, 2001). The discovery of increased lignin levels in *Bt* corn raises the question of whether other metabolic intermediates or products associated with the lignin and shikimic acid biosynthetic pathways have been affected by the transformation process. Aromatic biomolecules are extremely important in both plants and mammals as building blocks for hormones and other bioactive substances. The limited testing of these crops might easily have missed unintended increases or decreases in the levels of these other bioactive substances.

Finally, the finding that two completely different transformation events (MON810 and *Bt*11) are both associated with increased lignin levels raises an interesting question. Normally, one would expect that each non-repeatable, unique transformation event would yield unique unintended effects related to copy number, the site(s) of insertion, or other factors unique to the event. Finding the same unintended effect in two different transformation events suggests that the genetic transformation process *per se* (here, particle bombardment) might be responsible for an increase in lignin levels, and perhaps other undetected effects. Another possibility is that the cry1Ab gene or gene product exerts a lignin-promoting effect. The increased lignin content of *Bt* corn was brought to light only 5 years after market introduction. The lack of targeted testing for other bioactive substances associated with the lignin and shikimic acid pathways, and the failure to apply non-targeted techniques such as metabolic profiling and long-term animal feeding studies, highlight the serious gaps in the human health assessment of *Bt* corn.

SIMILARITIES AND DIFFERENCES BETWEEN *BT* SPRAYS AND *BT* CROPS

The EPA's chief justification for approval of *Bt* crops in the absence of crucial data is that *Bt* sprays have a history of safe use, and so *Bt* crops are presumed to be safe as well. This presumption is not justified for several reasons. First, it is reasonably clear that *Bt* sprays do cause allergic symptoms, as detailed at the beginning of this case study. Expert advisers to the EPA told the Agency that more studies are needed to determine the allergenic risk posed by Cry proteins in general – whether from *Bt* sprays or crops (SAP *Bt*, 2000). Secondly, there is likely much greater chronic exposure to Cry proteins in *Bt* crops than in sprays. Cry proteins in *Bt* sprays break down within several days to two weeks upon exposure to UV light (Ignoffo and Garcia, 1978; Behle *et al.*, 1997), while this is obviously not the case with *Bt* crops, which produce the toxin internally in grains and other plant tissues. Thirdly, *Bt* sprays are composed primarily of endotoxins in an inactive crystalline form. They are only toxic to insects with alkaline gut conditions that permit solubilization of the crystal to the protoxin, followed by proteolytic cleavage to the active toxin (Hilbeck *et al.*, 2000). *Bt* crops, on the other hand, are generally engineered to produce the *Bt* toxin (e.g. *Bt*11), which is active without processing, or a somewhat larger fragment (e.g. MON810). There is also evidence indicating that Cry toxins are more immunoreactive than Cry protoxins (Freese, 2001). Finally, the trend to increased Cry protein expression fostered by the EPA's "high-dose" strategy to slow development of pest resistance to *Bt* crops (EPA BRAD 2001e) may result in an increase in consumers' dietary exposure to *Bt* proteins. For instance, Mycogen/Pioneer's Herculex Cry1F corn, registered in 2001, expresses at least an order of magnitude more Cry protein in kernels than MON810 (Mendelsohn *et al.*, 2003). Use of chloroplast transformation, while still at the experimental phase, raises *Bt* protein levels still higher (Kota *et al.*, 1999). Thus, even if one ignores the evidence of allergenicity and

concedes that *Bt* sprays have a history of safe use, this is clearly not adequate grounds on which to judge *Bt* crops and their incorporated plant pesticides as safe.

BREAKDOWN IN THE REGULATORY SYSTEM

The question of whether *Bt* corn hybrids are harmful to consumers is still open. Testing along the lines indicated below is urgently needed to address this potential problem. However, even if no adverse effects were discovered, this case study dramatically illustrates the fundamental flaws in the US regulatory system for genetically engineered crops. Consider the following:

(1) the EPA registered, and in 2001 reregistered, Monsanto's and Syngenta's *Bt* corn events without following up on suggestive evidence of allergenicity, in particular, studies demonstrating Cry1Ab's amino acid homology to a known food allergen and stability to digestion;
(2) the EPA approved MON810 on the basis of studies that employed a derivative of a surrogate bacterial protein rather than the plant-produced protein;
(3) neither the EPA nor the FDA demanded characterization of the novel *Bt* fusion protein apparently produced by MON810;
(4) to our knowledge, there has been no published effort to investigate the potential health implications of a marked, unintended effect of the engineering process – namely, increased lignin levels in *Bt* corn stalks; and
(5) the FDA's flawed consultation document on MON810 reveals the fundamental weakness in its review practices.

Genetically engineered crops have been on the market for a decade, are planted on 58.7 million hectares worldwide (James, 2002), and have entered the diets of hundreds of millions, mostly without their informed consent. The unique risks posed by recombinant DNA technology applied to plants and the prevalence of foods containing ingredients derived from them demand adherence to extremely high standards of food safety. We have outlined some of the serious shortfalls in corporate testing procedures and US regulatory oversight for GE foods. Below we outline a testing regimen that we believe would better detect potentially harmful changes in GE foods and so better protect public health. While the manuscript was in preparation, a somewhat similar set of initial screening tests, in particular metabolic profiling, was proposed by Kok and Kuiper (2003).

Safety testing procedures

The previous paragraphs outline our concerns with an undefined and haphazard set of regulations and voluntary testing procedures that are applied to GE foods in the US They show that in many cases there is no testing of the plant product that is actually consumed. Instead, a bacterially produced surrogate protein is usually used. However, it is unambiguously clear that the inserted gene, when expressed in plants, directs the expression of a protein that can be modified in a large number of ways so as to render it distinct from the version made by bacteria (Schubert 2002). The expression of a foreign gene in a plant can also dramatically alter the metabolism of the host, resulting in the production of an altered array of gene products and low molecular weight metabolites (Roessner *et al.*, 2001). Our understanding of the science makes it clear that the genetic regulatory events resulting from the random insertion into the plant chromosome of a foreign gene driven by a viral promoter are going to be distinct from those caused by moving around linked blocks of genes through recombination or even increasing their number by chromosome duplication. At present, we do not understand the mechanisms of GE-induced changes in gene expression in sufficient detail to make an outcome prediction of the type that can be made when crossing two strains such as wheat that have been eaten safely for thousands of years. Even with

outcrossing to wild relatives, very few deleterious genes have been introduced into crops (Gepts, 2002). Since postmarket epidemiology is impossible in the absence of labeling, and genetic manipulations are essentially irreversible, we must get it right the first time. While US regulators, as outlined above, have made testing for potential health and environmental impacts optional and non-rigorous, the European Union, driven partly by informal public opinion, has adopted something akin to the precautionary principle. Perhaps the most extreme form of this concept was introduced by the French mathematician Blaise Pascal when he argued that even if you thought that it was very unlikely that a vengeful God existed, it was well worth your time and effort to behave as though he did, because making the extra effort for a short time to be good on earth would be much better than spending an infinity being tortured in hell. Therefore, European regulators argue that they are not prepared for the introduction of GE food until the long-term ecological and health consequences of these plants are better known, and they are willing to work a little harder to keep the public informed, for example, by requiring stringent labeling of GE products as well as the ability to trace the GE material to its origin (EC, 2003). In addition, it has been shown that the US regulatory system, based upon a weak interpretation of substantial equivalence (SE) that treats it as the end point rather than the starting point of evaluation, is substantially lacking in rigor and cannot be used to declare a product as safe as its conventional counterpart. It is therefore likely that many nations will require a more scientifically valid testing regimen than that used in the US What should these more rigorous tests look like? While we believe that the concept of SE is valid as a starting point, it clearly cannot be demonstrated merely with gross compositional analyses showing similar levels of protein, fat, starch, and perhaps selected nutrients and antinutrients in the GE and conventional plant, as in the US system. The transfection event used to create a GE plant generates unpredictable changes in gene expression that are going to be different in kind from those produced by traditional breeding. Therefore, testing must include screens for random changes in addition to the examination of potential problems that may be predicted from the expression of the transgene itself. The following paragraphs review some published test procedures and suggest a few additional testing criteria that should be useful in predicting the potential long-term health effects of a GE food.

To a large extent, many of the proposed schemes for testing GE foods suffer from the same erroneous assumption that is made by those who develop these products. That is that the insertion of a specific genetic sequence produces a phenotype that is related to, although perhaps somewhat divergent from, that produced by the gene in its normal host and cellular environment. While this may sometimes be the case, it is certainly not the rule, for totally unpredictable changes unrelated to the nature of the transgene can occur. This is because of the complexity of interactions between genes as well as the more obvious problems of gene disruption by insertion of the transgene itself. Unintended effects also arise with conventional breeding, but these usually occur in a limited and well-studied group of cultivars and are eliminated by backcrossing to make isogenic strains. Since GE plants may contain multiple insertion sites and chromosomal instabilities may result from the activation of dormant transposons (Meyer, 1999; Courtial *et al.*, 2001), unintended traits are not always inherited in a Mendelian manner, and productive backcrossing to yield genetically stable cultivars is difficult. Transposon activation also occurs during normal breeding, resulting in unpredictable gene insertions. This natural process, however, is very distinct from GE gene insertion. The transposed gene is not linked to a viral promoter to drive continuous expression and the GE insertion is strongly and artificially selected for in culture, while the transposon event in wild type plants is rare and subject to natural selection. Finally, sites of transposon insertion are not completely random throughout the chromosome, and may be quite distinct from the insertion sites of engineered genes. Therefore, while it is very important to determine the sequence of the inserted gene and gene product to identify possible allergenic sequences, potentially toxic fusion proteins and other novel products, it is also necessary and perhaps more efficient to use existing technology to initially do more global non-targeted screens for potential problems in three areas. These are screens for mutagens via the AMES test, for the introduction of toxic metabolic intermediates or the loss of nutrients by metabolic profiling, and for teratogenesis and other adverse effects by feeding experiments over several generations with laboratory animals. By establishing an accepted range of traits within a family of

cultivars in various environments, the introduction of the GE plant could be rapidly stopped if it falls outside of the normal distribution. A fourth screen, DNA chip analysis for gene expression, gives a good overview of changes in gene expression, and may be useful for the identification of specific toxins and antigens. However, at this point it has little additional predictive value as far as safety, and is available only for species where the genomic sequence is known, such as rice.

Of the first three screens, the AMES test is a very good predictor of the mutagenicity potential of a compound (Maron and Ames, 1983), and is a complement to the FDA requirement of long-term (2-year) carcinogenicity testing in animals for drug approval. This assay makes use of the fact that a nonvirulent strain of *Salmonella typhimurium* can grow in culture medium without amino acids. Defined mutants of the bacterium have been selected that require histidine for growth. Since carcinogens will cause mutations that reverse the original mutations, the carcinogenic potential of a compound or extract can be very simply assayed by the ability of the treated cells to grow on histidine-free medium. This assay has been adapted for assaying carcinogens with different specificities and is widely used throughout the world. It has been used extensively in the field of plant biology (Elgorashi *et al.*, 2003), but has not, to our knowledge, been used for GE food safety screening. It is simple and very inexpensive. Mutagenicity screening with the AMES test and the metabolic profiling discussed below would initially require baseline determinations of perhaps six widely planted cultivars of a particular crop, such as corn, including the parent of the GE line, grown under a variety of conditions. Once this is done and a distribution of mutagenic potential and individual metabolites is determined, using the part of the plant that is eaten, then it would not be necessary to repeat these assays. It is anticipated that a distribution of mutagenicity will be found, with each data point dependent upon the cultivar and the growth conditions. The GE crop would be grown under a similar set of conditions and its mutagenicity and metabolites characterized. If it falls within the normal distribution for toxic compounds, then it should be considered as passing the criterion; if not, it should be disallowed. A less permissive standard of comparison – perhaps the non-engineered isoline control – would be more appropriate for nutrients and other beneficial compounds.

Metabolic profiling is a process that uses the modern technologies of chromatography and mass spectroscopy to identify low molecular weight molecules made by cells, many of which are involved in normal metabolic processes such as energy metabolism (Trethewey *et al.*, 1999). However, plants make additional small molecules, such as the amino acids beta-N-oxalylamino-L-alanine (BOAA) from chickpeas and beta-methylamino-L-alanine (BMAA) from cycads, which can act as excitotoxins and cause serious neurological damage (Meldrum, 1993). It is the deregulation of the synthesis of low molecular weight toxins, mutagens and carcinogens caused by GE that has the potential to be the single greatest long-term health risk entailed by this technology. In addition, plants make a very large variety of nutrients and antioxidants whose loss or reduction could have serious adverse consequences for human health. Many of these can also be quantitated with metabolic profiling techniques (Roessner *et al.*, 2001; Schmelz *et al.*, 2003). Therefore, using a relatively small number of analytical procedures, it should be possible to quantitate many of the known nutrients, antioxidants, mutagens, carcinogens and toxins in a plant.

As alluded to above, essentially all plants naturally contain small but significant quantities of toxins, mutagens, and carcinogens (Ames *et al.*, 1990). Through many millennia of selective breeding, the levels of most of these noxious compounds have been minimized in our modern food crops. While it is not impossible to reactivate a toxin-producing pathway by normal breeding procedures (indeed, screening is always done in genera known to produce toxins), the unique technologies used to produce GE crops could activate dormant toxin pathways in species usually not associated with specific toxins. Therefore, the non-targeted approach for toxin screening should be more useful than trying to test for specific toxins based upon prior knowledge. Aside from the identification of toxins and nutrients, the majority of the information obtained from metabolic profiling may initially have no predictive value, but once large

numbers of both wild type and GE cultivars are examined it may be possible to identify patterns of metabolic changes caused by GE that will produce an undesirable phenotype. For example, there are metabolic stress responses in plants as there are in animals. Many of the products of these stress-response pathways are beneficial to both plants and humans. For example, phenolic antioxidants are frequently produced by plants in response to stress. The production by grapes of the potent antioxidant resveratrol in response to mold infection both kills the invading mold and promotes longevity in some species (Howitz *et al.*, 2003). If genetically engineering a plant were to trigger loss or reduction of this group of compounds, it could be identified and quantified by metabolic profiling.

An aspect of food safety testing which in our opinion has been grossly neglected is the use of animals. The lab mouse is the work horse of FDA drug screening programs, and is used to determine the safety of a product, particularly its effects on reproduction and development. No matter how much *in vitro* data are accumulated, it is impossible to determine if a product is safe unless it is tested in an animal. The FDA has long recognized this fact, and the plant biotech companies must also. The FDA requires an extensive, but not necessarily complex, series of safety tests to be performed, largely in mice, before any drug or even a food additive can be tested in humans. A few of these assays are easily adapted to testing plant material. In our opinion, the most critical tests are those for chronic toxicity, reproductive performance, and potential teratogenic effects by long-term feeding of the GE product, using the parental non-GE material as a control. It is frequently argued that it is hard to keep an animal healthy on a test diet and that the assay is irrelevant because people simply do not eat that much of a single food. The latter is clearly not true. According to Dr. Drinah Nyirenda, director of the Program Against Malnutrition in Zambia, a typical Zambian diet, for example, is 70% corn (Daily Democrat, 2003). The former problems can be circumvented by feeding the animals a balanced diet of which the test material is the major component, but not the only one. The goals of the chronic animal tests are to determine if any organs are susceptible to toxicity, to examine overall growth rate and health, and most importantly, to determine if the GE material has any effect on litter size (fertility) and other aspects of development (teratogenicity). These studies are critical because embryogenesis is an exquisitely fine-tuned process controlled by ultra-low levels of small molecules such as steroids and retinoids. Plants can make related molecules that may interfere with normal development. Over the past 10,000 years, it is likely that plant varieties that have adverse reproductive effects have been eliminated from our food supply, but modern GE technology may accidentally activate dormant pathways that adversely affect development. Feeding the GE plant to mice for a few generations would generate some assurance that this has not occurred.

The above paragraphs outline three non-targeted safety screening procedures that have not been extensively discussed in the context of GE food. A safety issue that has received more attention is the potential for genetic engineering to introduce novel allergens into food crops. The Edmonds Institute (1998) has proposed a series of tests to screen novel proteins for potential allergenicity. As discussed above, experts at the Food and Agriculture Organization and World Health Organization have also formulated an authoritative decision-tree testing protocol that involves structural comparison of the novel protein to known allergens, various *in vitro* tests (e.g. digestive and heat stability), and screening for IgE binding with sera from allergic patients (FAO-WHO, 2001). The importance of this particular protocol is that it represents the best thinking of international experts, serves as the basis for the authoritative *Codex Alimentarius* international food safety standards, and for the first time specifies detailed test parameters. As noted above in the case study, varying test conditions have given rise to widely divergent results for parameters such as digestive stability. Though testing in animals would be desirable to supplement *in vitro* testing, this must await development of a good animal model.

Many plant allergens remain unknown or uncharacterized. Nevertheless, it is widely agreed that the predicted amino acid sequence of novel transgenic proteins should be checked for sequence homology to all known allergens. FAO-WHO (2001) recommends overall sequence comparison as well as a stepwise comparison of 6-amino acid subsequences (based on minimum epitope length), with clear specification of

pass and fail criteria. Kleter and Peijnenburg (2002) recently applied FAO-WHO procedures to a group of 33 transgenic proteins in a two-step procedure designed to eliminate false positives. One transgenic protein that passed both cuts in their procedure was glyphosate oxidoreductase (GOX), a secondary mechanism for glyphosate resistance used in some varieties of glyphosate-tolerant canola and corn. It was found to have a subsequence that matched part of a proven allergenic epitope in a shrimp allergen (Kleter and Peijnenburg, 2002). Though not incorporated in the FAO-WHO protocol, Gendel (1998) argues persuasively for comparison procedures that allow for substitution of biochemically similar amino acids.

However, the remaining tests require protein, and it must be stressed that only protein produced by the part of the plant that will be eaten should be used, not a bacterially expressed surrogate protein, as is often done. Once again, FAO-WHO (2001) standards should be applied. Unlike earlier protocols, FAO-WHO specify the composition of simulated gastric fluid to be used for such tests (i.e. ratio of pepsin to test protein, pH) as well as breakdown evaluation criteria (i.e. how small must digested fragments be to qualify as "digested"). The FAO-WHO protocol also establishes procedures for testing novel proteins against IgE from individuals with known food allergies, with different sera testing procedures for GE proteins from source organisms with and without a known history of allergenicity. Nevertheless, the possibility that a previously unknown allergen can be introduced is a strong argument for labeling foods such that they can be traced to the point of origin. FAO-WHO also recommends consideration of postmarketing surveillance, in analogy to the final phase of drug testing, to capture allergic responses that may be missed with pre-market testing (FAO-WHO, 2001).

It seems to us that the safety testing procedures briefly outlined above – the Ames test for mutagenicity; metabolic profiling for toxic and nutritional compounds; extended animal feeding for carcinogenic, reproductive, teratogenic and other adverse effects; and allergenicity testing – should be sufficient, in conjunction with standard crop testing procedures, to determine if a new GE product falls within the accepted norm of safety of current food crops. All of the assays are straightforward, relatively inexpensive, and their uniform implementation would serve at least as a starting point for a rational testing regimen that may satisfy many science-based critics of this technology. Other scientific concerns stemming from the potential health risks of outcrossing and the expression of transgenes in different genetic backgrounds and growth conditions are more complex and have only recently been addressed (Haslberger, 2003). Obviously, the other ecological, political, social and economic issues surrounding genetically engineered crops are even more complex and will require a great deal more work to achieve a fair and equitable solution for all concerned.

Conclusion

In the preceding paragraphs, we have described the US regulatory system for GE foods, and with specific examples pointed out serious deficiencies in both regulatory oversight and corporate testing procedures. It is clear that the US regulatory process must be made mandatory, as well as more stringent and transparent. Any legal obstacles standing in the way of a thorough, mandatory, premarket review process must be overcome, with new statutes specifically designed for genetically engineered foods. Truly sound science must prevail in the debate over genetically engineered foods to ensure the safety of both consumers and the environment. The outline for an initial screening regimen proposed here offers an additional step toward this end.

Endnotes

[1] At the prompting of public interest groups and the Agency's scientific advisers, the EPA gave cursory treatment to four additional literature studies.
[2] In the US, this ill-chosen term, which seems to pre-judge the outcome of regulatory consideration, has come to replace the more neutral "review process."
[3] Recently renamed "plant-incorporated protectants." The EPA's role in regulation of antibiotic and herbicide resistance marker genes/proteins will not be addressed here.
[4] The Flavr-Savr tomato, engineered for longer shelf life, was subjected to a somewhat more stringent review only at the request of its developer, Calgene.

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(Note: Unpublished studies submitted to the EPA and identified with MRID numbers are available for inspection at the EPA at: Public Information and Records Integrity Branch (PIRIB), Room 119, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, Virginia, from 8:30 a.m. to 4 p.m., Monday through Friday, excluding legal holidays. The PIRIB telephone number is (703) 305-5805)

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