

# Genetically Modified Soybean in Animal Nutrition

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## 1. Introduction

In recent years, genetically modified (GM) plants, whose DNA has been changed using genetic engineering techniques, are mainly used as foods for human and feeds and foods for farm animals. To date, a number of GM products have been approved for human consumption but concerns over safety persist, mainly as regards either the detection of transgenic plant genes and proteins in animal systems or allergenicity and toxicity of GM plants.

Since their commercial release in 1996, the global cultivation area dedicated to the production of GM plants has increased significantly (ISAAA, 2010). The majority of GM crops currently produced, like soybean, corn, cotton and canola, have been engineered to enhance agronomic performance by transformation with genes encoding herbicide tolerance and pest resistance. GM soybean has been rendered tolerant to the glyphosate family of herbicides through expression of transgenic DNA from the CP4 strain of *Agrobacterium tumefaciens* that encodes 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS). Roundup Ready (RR) soybean have been grown commercially from 1996 and continued to be the principal biotech crop in 2010. Farm animals are currently fed soybean and soybean meal developed from genetic transformation as well as corn and corn products. The European Union imports soybean from USA, Brazil, and Argentina, the main users of biotech crops globally. About 90% of the compound feed produced in the EU contains GM soybean.

Although regulations with regard to GM plants have been developed primarily from the perspective of human consumption of GM food, it is generally assumed that these criteria are suitable for a risk assessment of the consumption of GM feed by livestock. The protocol for establishing “substantial equivalence” of GM plant compared to isogenic parental lines does not complete a nutritional safety assessment of a GM plant, rather, it provides a starting point for the overall assessment (FAO/WHO, 2000). Based on the European novel food and feed regulation, all foods and feeds containing or derived from approved GM products in amounts greater than a 0.9% threshold are subject to labelling rules (European Commission, 2003). Labelling of feeds containing GM ingredients gives farmers the choice of using such feed for their livestock. However, products such as milk, meat, and eggs, that are derived from livestock fed transgenic feeds are exempt from EU-labelling laws. Several studies have been conducted to evaluate the safety of GM crops, but there is still a debate on the risk of GM consumption and their potential passage into tissues.

Current researches suggest that the passage of plant DNA fragments across the intestinal barrier is a natural event, as demonstrated by the detection of endogenous, high copy number chloroplast genes from plants in several animal tissues and products. Low copy endogenous and transgenic DNA in animal tissues have been detected but to a lesser extent than high copy genes.

For several years, no direct evidence that GM food may represent a possible danger for health has been reported and the scientific literature in this field is still quite poor, especially as to the possible effect of a diet involving a significant amount of GM plants. More recently, a number of papers have been published and controversial results have been obtained. However, some have found significant modifications in some nuclear features in mice fed GM soybean and, more recently, it has been reported that the activity of some enzymes was altered in rabbit and goats fed GM soybean, as confirmed also by histochemistry which showed a widespread distribution of enzyme activity in myocytes, myocardiocytes, epithelial cells of renal tubules and hepatocytes. These observations suggest that the risk of genetically modified crops cannot be ignored and requires further investigations in order to identify possible long-term effects of GM plants on both livestock and human consumption. The main focus of this chapter concerns the genetically engineered soybean, its effects on human and animal health, the productivity of this GM crop and the outcome for environment.

## **2. Agronomic impact of genetically modified soybean**

Genetic engineering has been widely applied to agriculture to obtain specific plant characteristics which can lead to an improvement in both food quality and yield. Compared with traditional plant breeding methods, such as artificial crossing or hybridization, biotechnology now allows for the introduction of DNA from outside the plant kingdom. Selective inclusion of single or multiple traits can be performed to change the quality of agricultural crops. According to statistics released by the International Service for the Acquisition of Agri-Biotech Applications (ISAAA, 2010), the area of planted transgenic crops was 148 million hectares in 2010, a approximately 87-fold increase from the 1996 level (1.7 million hectares of biotech crops). The number of countries adopting biotech crop cultivation has increased crops consistently from 6 in 1996 to 29 in 2010. The Unites States (US), followed by Brazil, Argentina, India, Canada, and China continued to be the principal adopters of biotech crops globally, with 66.8 million hectares planted in the US.

The majority of genetically modified (GM) crops currently produced have been engineered to enhance agronomic performance by transformation with genes encoding herbicide tolerance or pest resistance. From the first commercialization of biotech crops in 1996, to 2010 herbicide tolerance has consistently been the dominant trait. In 2010, herbicide tolerance deployed in soybean, corn, canola, cotton, sugarbeet and alfalfa, occupied 61% or 89.3 million hectares of the global biotech area. In 2010, the stacked double and triple traits occupied a larger area (22% or 32.3 million hectares) than insect resistant varieties (26.3 million hectares) at 17%. The insect resistance trait products were the fastest growing trait group between 2009 and 2010 at 21% growth, compared with 13% for stacked traits and 7% for herbicide tolerance.

Biotech herbicide tolerant soybean continued to be the principal biotech crop in 2010, occupying 73.3 million hectares or 50% of global biotech area, followed by biotech corn (46.8 million hectares at 31%), biotech cotton (21.0 million hectares at 14%) and biotech canola (7.0 million hectares at 5%) of the global biotech crop area.

Farm animals are currently fed soybean and soybean meal developed from genetic transformation as well as corn and corn products such as corn gluten feed and meal. Europe is strongly dependent upon the American continent for its protein requirements amounting up to 90 to 95% for soybean, 40 to 60% for corn derivatives and partly for canola grain or meal (Aumaitre, 2004).

### 3. Genetic modification of soybean

Traditionally, plants with desirable characteristics were chosen for food of the next generation. The desirable characteristics arose from naturally occurring variations in the genetic make-up of individual plants. Unlike conventional genetic modification that is carried out through time-tested conventional breeding of plants as combining genes from different organisms is known as recombinant DNA technology and the resulting organism is said to be genetically modified, or genetically engineered or transgenic (Pandey et al., 2010).

Transgenic plant is one that has received a segment of DNA or genes from another organism (known as heterologous or foreign DNA) using recombinant DNA techniques. The foreign DNA is integrated through natural systems present in plant cells into the plant's genome. The newly introduced genes are subsequently inherited in a normal Mendelian manner through pollen and egg cells. The mainly process of introducing DNA into plants (called transformation) uses the *Agrobacterium* mediated method and it can be achieved both in monocotyledonous plants such as wheat, barley and rice and in dicotyledonous plants such as soybean, potato and tomato.

The soil bacterium *Agrobacterium tumefaciens* causes crown gall disease on some plants, in particular in dicotyledonous species. In causing crown gall disease *A. tumefaciens* transfers DNA (the transferred DNA or T-DNA) from the bacterium to the plant. In nature the transferred bacterial DNA cause the symptoms associated with crown gall disease. In the early 1980s scientists removed the disease causing genes from this bacterium and the T-DNA is now routinely used to transport foreign genes into plants. *Agrobacterium* cells carrying the foreign genes of interest are incubated with cultured cells of the recipient crop plant and transgenic plants are regenerated from them. Not all cells subjected to this process are successfully modified so it may be necessary to identify the modified cells using marker genes which are closely linked to the genetic material that is transferred. These selectable marker genes usually confer resistance to an antibiotic such as kanamycin or resistance to an herbicide.

The genetically modified soybean (named RoundUp Ready, RR) has been rendered tolerant to the glyphosate family of herbicides through expression of transgenic DNA from *Agrobacterium tumefaciens* sp. strain CP4 that encodes 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS). The CP4 EPSPS protein expressed in GE glyphosate tolerant plants is functionally equivalent to endogenous plant EPSPS enzymes with the exception that CP4 EPSPS displays reduced affinity for glyphosate (Franz et al. 1997). This soybean is, also, composed of a 35S promoter from cauliflower mosaic virus (CMV) and a NOS-terminator, a terminator of nopaline synthase gene.

According to the Center for Environmental Risk Assessment (2010), the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS: EC 2.5.1.19) family of enzymes is ubiquitous in plants and microorganisms. EPSPS enzymes have been isolated from both sources, and their properties have been extensively studied. EPSPS proteins catalyze the

transfer of the enolpyruvyl group from phosphoenol pyruvate (PEP) to the 5-hydroxyl of shikimate-3-phosphate (S3P), thereby yielding inorganic phosphate and 5-enolpyruvylshikimate-3-phosphate (Alibhai and Stallings, 2001). Shikimic acid obtained is a substrate for the biosynthesis of aromatic amino acids (phenylalanine, tryptophan and tyrosine) as well as many secondary metabolites, such as tetrahydrofolate, ubiquinone, and vitamin K. Importantly, the shikimate pathway and, hence, EPSPS proteins, are absent in mammals, fish, birds, reptiles and insects (Alibhai and Stallings, 2001). In contrast, it has been estimated that aromatic molecules, all of which are derived from shikimic acid, represent 35% or more of the dry weight of a plant (Franz et al. 1997).

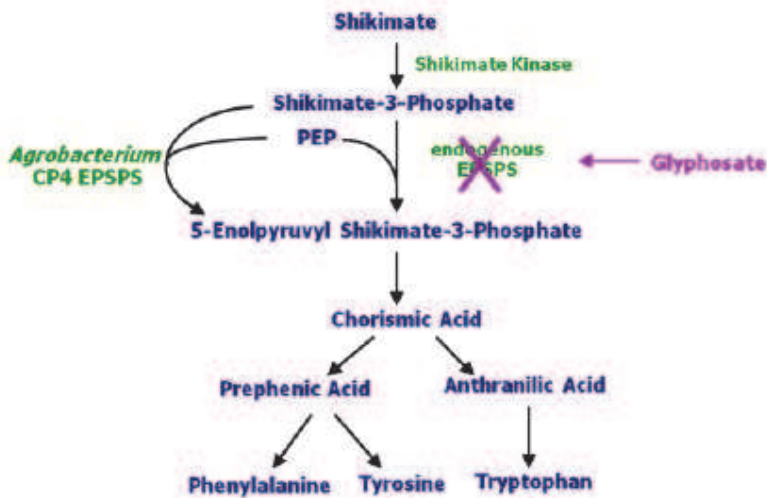


Fig. 1. Schematic representation of glyphosate mode of action and mechanism of CP4 EPSPS mediated tolerance (Center for Environmental Risk Assessment, 2010).

#### 4. Nutritional assessment of genetically modified soybean

In animal nutrition, many studies with GM plants were carried out in target species using the substantial equivalence method. The application of this method to animal studies led to the development of the concept of nutritional equivalence which implies specific measurements regarding animal production. The European Commission has a combined safety approach that requires an assessment of risks for humans, animals, and the environment prior to approval of importation or cultivation of a novel crop (European Commission, 2001). An integral part of the safety evaluation of GM plants is to test for “substantial equivalence”. The concept of substantial equivalence is the starting point and guiding concept for safety assessment (Food and Agriculture Organization/World Health Organization, 2000). It is not the conclusion, but it is part of the safety assessment (Konig et al., 2004; Kuiper & Kleter, 2003). The aim of such a test is to determine whether a transgenic plant is substantially equivalent to its conventional counterpart at a chemical and nutritional level. While the parameters to be measured have not been formally defined, minimal analyses performed should determine whether the major nutritional components (i.e., lipids, carbohydrates, proteins, vitamins, minerals, trace elements) and known antinutrients and

toxins of transgenic plants are equivalent to those in conventional varieties that have a history of safe use. Guidelines have been established by several organizations regarding assessment of the allergenic risk of each novel protein expressed in a GM plant, prior to market approval (FAO/WHO, 2000; König et al., 2004; Martens, 2000). These typically include comparison of amino acid sequence homology of the novel protein to known allergens and digestion of the protein in simulated gastric environments. While allergic reactions are primarily a concern for human consumption of GM foods, certain proteins in soybean have been shown to elicit allergic reactions in calves and piglets. The assessment of the safety of GM organisms addresses both intentional and unintentional effects that may result as a consequence of genetic engineering of the food source. Future transgenic crops are expected to contain fewer or no marker genes in the final products since marker free insertion techniques or methods to eliminate marker genes from transgenic plants are being improved. The assessment of safety measures are a lengthy and tedious process (Figure 2). The nutritional aspects, risk characterization and exposure assessment are preliminary steps being taken. Before hitting the market, all GM products have to pass all the allergic tests and provide the details. Only those products found as possessing no harmful or allergic effects are only recommended.

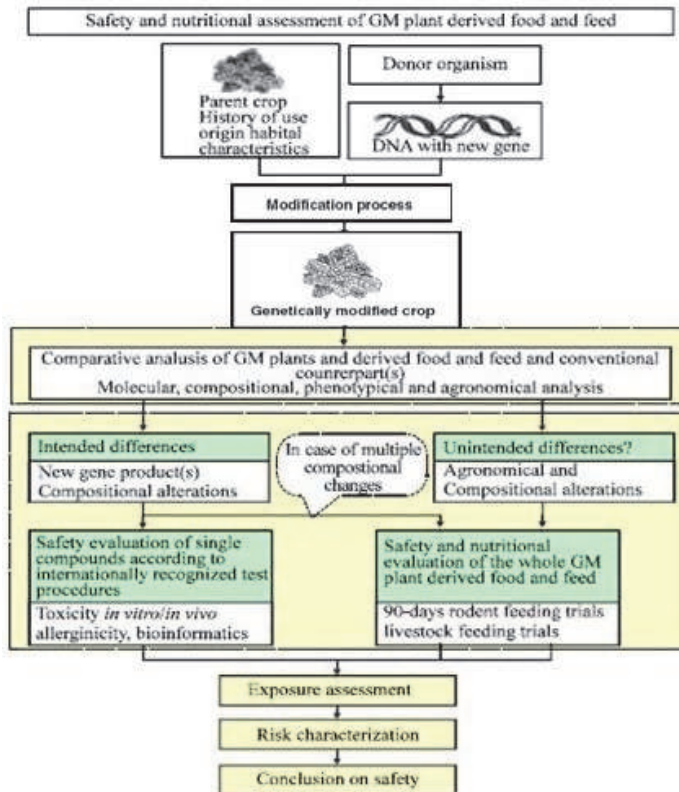


Fig. 2. Pre-market safety and nutritional testing of genetically modified plant derived food and feed (EFSA, 2008).

In most countries authorities and agencies involved in feed/food safety assessment have based their safety assessment strategies and guidelines on this approach. To provide consumers the opportunity for choice, in 2004 the European Union (EU) has extended regulations concerning GM foods to include animal feeds and feed additives. According to Regulation (EC) No. 1829/2003 (European Commission, 2003), all foods and feeds containing or derived from approved GM products in amounts greater than a 0.9% threshold are subject to labelling rules. Labelling of feeds containing GM ingredients informs farmers and gives them the choice of using such feed for their livestock. However, products such as milk, meat, and eggs, that are derived from livestock fed transgenic feeds are exempt from EU-labelling laws. One of the controversies, important for safety aspects of GM feeds, is a potential possibility of transfer of the transgenic DNA to animal tissues, and in consequence its negative effect on consumers of such products originating from animals fed diets containing GM plants. Detailed studies of the feeding qualities of GM plants for livestock and their nutritional evaluation have been reviewed previously (Aumaitre et al., 2002; Flachowsky et al., 2005a; Flachowsky & Aulrich, 2001). A lot of animal studies with GM plants aimed to evaluate the compositional and nutritional equivalency of transgenic feeds and their conventional counterparts.

Accordingly to the substantial equivalence theory, feed chemical analyses were performed to determine whether the macronutrients, vitamins, minerals and even trace elements were found at the same level as in the conventional or isogenic plants. The composition studies confirmed the substantial equivalence of genetically modified (GM) soybean to conventional counterpart (Table 1) (Cromwell et al., 2001; Padgett et al., 1996).

	Conventional soybean	GM soybean
Dry Matter	90.30	91.00
Crude Protein	51.50	51.20
NDF	4.95	4.85
Lysine	3.16	3.09
Methionine+Cysteine	1.47	1.51

Table 1. Chemical composition (% dry matter) of genetically modified soyabean (RoundUp Ready, RR) and its conventional counterpart (Adapted from Cromwell et al., 2001). NDF: neutral detergent fiber.

The concerns that have been raised with respect to the potential risk associated with the use of GM plant products in animal feed are related to the possible unintended effects of inserting novel DNA into the plant by biotechnology. The possible “side effects” of the genetically modification is often termed unintended effects and may result from the random integration in the genome of the novel DNA which may result in an over-expression in the plant of inherently toxic substances such as anti-nutritional factors (ANFs), silencing of endogenous plant genes (e.g essential nutrients), or alterations in host metabolic pathways (Novak & Hasleberger 2000; Saxena & Stotzky, 2001). The possible combination of an unexpected increase in expression of endogenous ANFs and the presence of new exogenous toxicants have been of particular concern as these could compromise the quality of feedstuffs and may affect animal health and nutrition (Francis et al., 2001). Studies also include any known anti-nutritional factors (ANFs), such as trypsin inhibitors in soybean,

interfering with nutrient absorption or natural toxicants typically present in the genus (Padgette et al., 1996). Trypsin inhibitors are similarly destroyed during heating associated with oil extraction and preparation of soybean meal. In addition, the activity of urease measured by the variation in pH is efficiently reduced by heat treatment whatever the genotype of the soybean kernel (Table 2).

	Parental soybean		RR	
	Raw	Meal	Raw	Meal
Lectins (HU/mg sample)	1.2	-	1.0	-
Trypsin inhibitor (TIU/mg sample)	22.6	3.4	23.7	3.3
Urease (pH)	2.18	0.03	2.17	0.04

Table 2. Main anti-nutritional factors in raw soybean and soybean meal (parental vs. RR): absence of effect of genetic modification for glyphosate resistance (Adapted from Padgette et al., 1996).

Additional nutritional data can be of importance in the case of oil seeds or oil-rich cereals such as corn because they can markedly affect the composition of fatty tissues when fed to farm animals. Data obtained from the analysis of corn kernels demonstrated similar proportions of fatty acids in oil of kernels of insect resistant and herbicide-resistant corn. Similarly, and in the majority of cases, it has also been observed that insect resistant and herbicide-tolerant corn kernels contain similar proportions of amino acids. The introduction of Bt and herbicide tolerance genes in corn has never been found to create starch modifications expressed as the proportion of amylose and amylopectin. Thus similar proportions of 21.5 and 21.0% of amylose have been found in Bt and herbicide-tolerant modified corn, respectively, compared to 22.4 and 22.7% of amylose in starch of the isogenic varieties, respectively (Benetrix, 2000). Data from the literature have many times corroborated the substantial equivalence in major nutrients and minerals and trace elements in corn and kernels of GM compared to isogenic control corn (Brake & Vlachos, 1998; Sidhu et al., 2000). The whole modified corn plant (Clarke & Ipharraguerre, 2001; Faust, 2000) has also been found to be substantially equivalent in composition to isogenic plants. All these data suggest a similar nutritional value for the feed material derived from the modified plants (Aumaitre et al., 2002).

In order to evaluate the nutritive value of feeds for ruminants, nowadays the *in vitro* gas production technique (Theodorou et al. 1994) (IVGPT) is commonly used. IVGPT is based on the assumption that the accumulated gas production by a substrate, incubated in with rumen liquor, is proportional to the amount of digestible carbohydrates, and thus highly correlated to the energy value of feeds. In addition, IVGPT allows to study also the fermentation kinetics of feeds. Tudisco et al. (2004) in a research, aimed to compare the fermentation kinetics of Roundup Ready defatted soybean to its conventional counterpart by the IVGPT, found that the genetic modification, although did not affect the chemical composition, led to a significantly lower cumulative gas production and volume per gram of incubated organic matter. It could be hypothesized that the genetic modification may lead to pleiotropic effects (effect of a single gene on multiple phenotypic traits) that may alter the starch and/or protein structure. Alternatively, the results could be explained with a plant DNA transfer to ruminal bacteria which may modify their fermentation activity.

In any case, the results of this research arouse concerns in term of food safety, because other unpredictable metabolic effects, such as metabolic interferences, or direct or indirect insertional mutagenesis cannot be excluded. With this regards Seralini et al. (2011) report that by insertion of the transgene in varieties producing Cry1Ab toxin caused a complex recombination event, leading to the synthesis of new RNA products encoding unknown proteins, or/and to metabolic pathways variations which caused up to 50% changes in measured osmolytes and branched aminoacids.

#### **4.1 Nutritional testing of GM feed with GM soybean in target animal species**

Many studies with GM plants were carried out in target species to assess the nutritive value of the feed and their performance potential and were revealed no significant differences in performance indices and quality parameters of meat, eggs or milk, when farm animals were fed diets containing GM or conventional feeds (Aumaitre et al., 2004; Flachowsky et al., 2005a; Świątkiewicz S. & Świątkiewicz M., 2009). These studies have focused on livestock nutrition, in order to confirm nutritional equivalence and to obtain further information concerning the safety of animal products.

As regards monogastric livestock, many feeding studies with 1-day-old broiler chicks have been reported (EFSA, 2008), including GM lines of corn, soybean, canola and wheat and appropriate counterparts. Few experiments are available with laying hens (Aulrich et al., 2001; Halle et al., 2006) where GMOs were compared with near isogenic counterparts. McNaughton et al. (2011) compared the nutritional performance of laying hens fed corn grain (event DP-Ø9814Ø-6) and processed soybean meal (event DP-356Ø43-5), individually or in combination, with the performance of hens fed diets containing conventional corn and soybean meal. The performance (body weight, feed intake and egg production) and egg quality of hens fed GM feeds was comparable with that of hens fed diets formulated with conventional feed. In each study, chemical composition and nutritional value of GM lines and the near-isogenic non-GM lines were found to be comparable without biologically significant differences in the production parameters measured. Experiments with growing and laying quails were carried out to test diets with isogenic or GM Bt 176 corn (Flachowsky et al., 2005b; Halle et al., 2006). Health, hatchability and performance of quails and the quality of meat and eggs were unaffected by the diets. Improvements in livestock performance were noted significant with the diet containing Bt corn compared to diets containing conventional corn grain (Piva et al., 2001a, 2001b). The authors attributed the results to the fact that the use of Bt lines reduced secondary fungal infection and, as a consequence, reduced mycotoxin contamination. Research conducted with growing and finishing pigs (EFSA, 2008) including GM corn grain, sugar beet, soybean meal, rapeseed meal, rice and wheat, showed that when compositional analyses of GM lines and the near-isogenic non-GM line and commercial varieties were comparable, then nutritional equivalence was also established.

As regards ruminants, comparable performance of beef cattle fed corn grain, corn silage or stover from GM plants or from conventional plants is reported, and in dairy cows the inclusion in the diet of feed ingredients derived from a wide range of GM plants unaffected feed intake, milk yield and composition (EFSA, 2008). Milk quality is generally measured as the fat, protein and lactose concentration and as such there is no evidence to suggest that the inclusion of GM feed ingredients affects milk quality. As with other livestock species, studies with lactating dairy cows also showed that once compositional equivalence was demonstrated then nutritional equivalence occurred.



Finally, the production studies carried out with fish provided similar conclusions to those drawn from studies conducted with other livestock species (EFSA, 2008).

	Parameters	Results	References
Poultry	BWG, ADG, FCR, muscle weight	No significant differences	(Hammond et al., 1996)
Pig	BWG, DMI, FCR, carcass quality, sensory score of meat	No significant differences	(Cromwell et al., 2001)
Cow	Milk yield and composition, DMI, nitrogen balance, rumen VFA composition	No significant differences	(Hammond et al., 1996)

Table 3. Some experiments carried out to establish the qualitative and quantitative performance of monogastric or ruminant livestock fed with GM soybean. BWG: Body Weight Gain; ADG: Average Daily Gain; FCR: Feed Conversion Ratio.

## 5. Fate of transgenic DNA and new protein in animal organs and products

One of the most important questions about the use of GM products in animal nutrition is the possibility that modified DNA could be transferred from plants to animal products or to bacteria, with harmful consequences (FAO, 2004). Other problems regard the ability of transgenic proteins to provoke food intolerance or allergic reaction in susceptible people. Hence, it is necessary to consider the destiny of these molecules within the animal organism (Alexander et al., 2007).

The gastrointestinal tract is constantly exposed to DNA that is released from partially or completely digested food, ingested microbes, and DNA from intestinal microflora. Ingested food is mechanically disrupted and the released DNA, although poorly digested, is cleaved through acid hydrolysis and enzymatic digestion into small DNA fragments. Eventually some of these fragments are converted to single nucleotides. Acid hydrolysis in the gastrointestinal tract is expected to depurinate most adenosine and guanine nucleotides of the food DNA (Klinedinst & Drinkwater, 1992). The presence of various phosphatases and deaminases continue to destroy the structural integrity of any free DNA. The breakdown products of DNA are absorbed for using at the cellular level for synthetic processes as they may be found in blood and tissues (McAllan, 1982). All though there were conflicting reports on the fate of GM DNA in the biological system it was observed that DNA could pass through the gut wall into the blood stream and taken up by cells in the blood, liver, spleen and passed through the placenta to the cells of the foetus and the newborn one (Doerfler & Schubert, 1998).

In ruminants, experimental evidence suggests that more than 80% of DNA is completely disrupted after 2 hours (Wiedemann et al., 2006). However, this degradation is not complete and not immediate (van den Eede et al., 2004).

In animal tissues some fragments of chloroplast DNA have been found. The reason why chloroplast DNA is more frequently detected in animal products is the number of the genes involved and the sensitivity of the PCR method. In transgenic plants, every cell contains hundreds chloroplast genes, but only one transgenic gene (Aumaitre et al., 2002).

Research on the fate of foreign DNA in the mammalian organism showed that PCR products specific to foreign DNA could be detected therein. It was concluded that DNA fragments from the gastrointestinal tract could reach the bloodstream and be transported through the epithelium of the gut and the cells of the Peyer's patches to spleen and liver cells. Such DNA fragments are probably retained for a short while and then digested (Schubbert et al., 1994, 1997, 1998). While the intestinal tract does not seem an absolute barrier against the uptake of macromolecules or even of microorganisms, the mechanism of foreign DNA uptake by the intestinal wall epithelia is unknown. In addition, not much is known about the degradation and integration of the DNA. There is some evidence that fragments of foreign DNA are not digested in the gut and might enter the organism or become incorporated into cells lining the gut wall (Doerfler, 2000; Tony et al., 2003).

Transfer of the plant DNA to bacteria needs several steps, and the expectation seems to be extremely low (Kuiper et al., 2003; Sharma et al., 2004). Bacterial resistance to antibiotics is not a specific problem of genetic engineering. According to Directive 2001/18/EC, use of GMOs containing antibiotic-resistant genes will be forbidden starting from 01/01/2009 (European Commission, 2001).

Data are also available on the fate of recombinant plant DNA in the gastrointestinal tract of humans. By *in vitro* simulation of human digestion, 80% of the transgene in naked GM soybean DNA was degraded in the gastric simulations, while no degradation of the transgenes contained within GM soybean and corn was observed in these acidic conditions (Martin-Orúe et al., 2002). In the small intestinal simulations, transgenes in naked soybean DNA were degraded. In contrast, the corn nucleic acid was hydrolysed in the small intestinal simulations in a biphasic process in which approximately 85% was rapidly degraded, while the rest of the DNA was cleaved at a low rate of degradation.

The number of transgene copies passing to the small intestine of human ileostomists consuming GM soya were successfully quantified, and up to 3.7% of the transgene could survive passage (Neterwood et al., 2004).

Finally, another factor that will directly affect gene persistence throughout the digestive tract and therefore indirectly affect the chance of passage across the GIT epithelium is the digestibility of the ingested plant species. Feedstuffs with relatively greater digestibility, such as soybean meal, are likely to have their DNA degraded more rapidly, decreasing the chance of absorption.

A low copy endogenous (soybean lectin) and recombinant (CP4 epsps) gene in longissimus dorsi muscle samples from pigs fed herbicide-tolerant soybean meal, in the grower and finisher phases, respectively were attempted to detect (Jennings et al., 2003). The same results about the fate of the CP4 epsps gene in other species were reported (Klotz & Einspanier, 1998).

The CP4 epsps transgenic gene was not found in muscle and liver of chicken fed herbicide-tolerant soybean up to 7 weeks after ingestion (Khumnirdetch et al., 2001). According to the authors, GM soybean fragment were degraded in the gastrointestinal tract.

As regards ruminants, chloroplast gene fragments were found in the leucocytes of dairy cows fed small quantities of transgenic soybean meal, while no fragments of the transgenic DNA were found in any tissue examined and in milk (Klotz & Einspanier, 1998). Similarly, high copy chloroplast "rubisco" gene fragments were found in the blood of cattle fed GM and soybean meal, but transgenic sequences were never detected (Phipps et al., 2003).

In fish, soybean meal is used as dietary source of protein in their diet, however, because of the presence of anti-nutritional factors (ANFs) their inclusion levels should be kept low (Olli

et al., 1994). The fate of ingested GM soybean DNA fragments (120 and 195 bp) and a 180 bp fragment of the lectin gene of soybean in Atlantic salmon and their survival through the gastrointestinal tract (GIT) were investigated and the DNA was traced in a variety of fish tissues (liver, muscle and brain) (Sanden et al., 2004). Only the smaller GM DNA fragment (120 bp) was amplified from the content of the stomach, pyloric region, mid intestine and distal intestine, while no transgenic or conventional soybean DNA fragments were detected. The uptake of dietary DNA into blood, kidney and liver of salmon was investigated also by other authors (Nielsen et al., 2005) which determined the DNA fragment size if dietary DNA was detected. Most of the feed (partially digested) was found in the pyloric region, mid intestine, and distal intestine at 4, 8, and 16 h after force-feeding, while the highest concentrations of dietary DNA in liver and kidney were found 8 h after force-feeding, and blood up to 64 h. Finally, the cauliflower mosaic virus 35S promoter fragment (220 bp) of the GM defatted soybean meal was detected in the muscle of rainbow trout receiving both levels of GM soybean (approximately 15 and 30%) diet by nested PCR, but the frequency of detection was greater at the higher inclusion level (Chainark et al., 2006). Additionally, the promoter fragment was not detected by the fifth day after changing the diet to non-GM soybean. Conversely, the promoter fragment was not detected from fish fed with the non-GM SBM diet. Successively, Chainark et al. (2008) traced foreign DNA fragments from genetically modified defatted soybean meal (GM SEM) in rainbow trout by nested polymerase chain reaction (PCR) and located by in situ hybridization. Either a GM or non-GM SBM formulated diet (42% protein) was fed to fish (average weight 50.5 g) for 2 weeks. The degradation results showed that the cauliflower mosaic virus 35S promoter (220 bp) fragment was detected in the contents of digestive system only in fish fed the GM SBM diet, and it was not detected on the third day after changing the diet to the non-GM SBM diet. For the possible transferral results, the promoter fragment was detected in the leukocyte, head kidney and muscle only of fish fed the GM SBM diet; it was not detected on the fifth day after changing the diet to the non-GM SBM diet. These results suggest that a foreign DNA fragment was not completely degraded and might be taken up into organs through the gastrointestinal tract. However, foreign DNA was not detected after the withdrawal period. Thus, the data show that uptake of DNA from GM SBM might not remain in the tissues of fish fed GM SBM diet. Similarly, Ran et al. (2009) found DNA fragments from RR soybean in different tissues and organs of tilapias (*Oreochromis niloticus*, GIFT strain).

Tudisco et al. (2006) in order to evaluate the presence of plant DNA fragments in tissues to follow the fate of plant fed, carried out a research on twenty weaned 30-day-old New Zealand rabbits (10 males and 10 females), individually caged, which were equally assigned to control (C) and treated (T) groups. The animals were given a diet containing soybean meal (solved extracted) which was from conventional or Roundup Ready beans, for group C and T, respectively. The presence of chloroplast DNA was found in tissues and blood from both control and treated groups. The percentage of positive samples were: 50% (blood), 70% (muscle), 80% (heart), 70% (liver) and 80% (kidney). By contrast specific fragments of soybean were not detected in all samples but only in the plant samples. Similarly transgenic fragments gave undetectable results.

Subsequently, the same authors (Tudisco et al. 2010) investigated the presence of DNA fragments in blood and milk from goats fed conventional (control) or Roundup Ready soybean and in blood, skeletal muscle and organs from their offspring. Transgenic target DNA sequences (35S and CP4 EPSPS) were detected in blood and milk from goats that received a diet containing transgenic soybean as well as from some samples of their

offspring, not in the control group. Those findings show plant DNA fragments are likely to survive digestive processes to some extent (Duggan et al., 2003; Einspanier et al., 2004), as well as their transfer to blood and milk. In addition, the detection of plant DNA in tissues and organs of nursed kids could support the hypothesis of a gene transfer through milk.

## 6. Effects on animal health of GM soybean

In different experiments food and feed derived from GM plants, mixed in animal diets have been fed to rats, mice or other animal species during different periods of administration, and parameters such as body weight, feed consumption, blood chemistry, organ weights, histopathology, etc., have been measured. With respect to recent studies on safety assessment of GM soybeans, the scientific literature shows rather contradictory results.

No immunotoxic activity or an increase in the IgE in serum and histopathological abnormalities were found in the mucosa of the small intestine of rats and mice fed heat-treated GM soybean meal containing the cp4-epsps (Teshima et al., 2000).

In Sprague-Dawley rats, Appenzeller et al. (2008) conducted a subchronic feeding study with the herbicide-tolerant soybean DP-356043-5 (356043). Diets were fed to young adult animals for at least 93 days. Compared with rats fed with 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 microscopic pathology. Similarly, Delaney et al. (2008) carried out in Sprague-Dawley rats a subchronic feeding study of high oleic acid soybeans (Event DP-305423-1). DP-305423-1 (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. 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.

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.

Finally, 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 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 with 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, immunocytochemical 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.

Battistelli et al. (2010) investigated the duodenum and colon of mice fed on genetically modified (GM) soybean during their whole life span (1–24 months) by focusing their attention on the histological and ultrastructural characteristics of the epithelium, the

histochemical pattern of goblet cell mucins, and the growth profile of the coliform population. Even if the GM soybean-containing diet did not induce structural alterations in duodenal and colonic epithelium or in coliform population, the histochemical approach revealed significant diet-related changes in mucin amounts in the duodenum. In particular, the percentage of villous area occupied by acidic and sulphomucin granules decreased from controls to 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, all these studies 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.

Evaluating the GM soybean in Atlantic salmon diet, enlarged spleen and possible impaired spleen function as the number of smaller-sized red blood cells simultaneously increased were indicated (Hemre et al., 2005).

The same authors (Sagstad et al., 2008) reported lower plasma triacylglycerol levels and a significantly larger spleen somatic index in fish groups fed GM soybean compared to groups fed non-GM soybean.

Ermakova (2006) examined the effect of glyphosate-resistant (RR) GM soybean seeds fed to pregnant female rats on the number and weight of pups delivered. The study was originally published in Russian, and was heavily criticised for using coated seeds ready for planting instead of beans suitable for feed. The control non-GM soybean was not the isogenic parent line, either. However, because of the possible serious implications of the results of this study for humans and animals it should have been repeated and possibly verified by other scientists with the correct GM soybean diets. Indeed, she has repeatedly pleaded for this but no one dared to try to reproduce her experiments. In this study rats were fed with laboratory rat chow and this diet was complemented with GM or conventional soybean for

two weeks before mating, during the pregnancy and during suckling and the body mass and the number of pups were observed. The data indicated that on the GM soybean-supplemented rat chow significantly fewer pups were born, and with smaller body mass, than on the control non-GM soybeans.

In order to evaluate the possible health effects of a GM diet, Tudisco et al (2006) studied the activity of organ specific enzymes in two groups of New Zealand rabbits, given a diet containing soybean meal which was from conventional or Roundup Ready beans.

Statistical differences were detected in kidney for alanine aminotransferase (ALT), gamma glutamyltransferase (GGT) and lactic dehydrogenase (LDH) (higher activity for group fed GM soybean) whereas in the heart such result was seen only for LDH. No statistical differences were found for serum, liver and skeletal muscle. Significant differences between groups were detected for heart LDH1 and LDH2 and for kidney LDH1, thus confirming the significant increase of the enzyme in these tissues. Moreover, despite no significant differences were found for LDH total activity in liver, a significant increase (LDH1) and decrease (LDH4) were found also in this organ.

Brasil et al. (2009) found that rats fed on GM soy showed altered morphology of the uterus and the ovaries: had greater volume density of endometrial glanular epithelium, reduced follicle number and increased corpus luteum numbers (a tendency to abort or less of a chance to get pregnant). Although the GM diet was not supplemented with cysteine as the other diets, and it is difficult to assess if the results were due to consumption of the transgenic soy itself or were due to the presence of glyphosate (and/or AMPA), always present in GM seeds, the findings are disturbing and warrant further studies.

A recent study found that the hamsters fed with GM Soybean showed the growth of hairs inside the pouches of the mouth and the number of hairy mouthed hamsters was much higher in the third generation of GM soy fed animals than in others (Baranov et al., 2010). According to the authors, it remains unclear why these hair structures appear in the oral cavity of mammals. We may only speculate on the origin of this phenomenon. The gingival pouches may result from paradontitis and paradontosis caused by feeding on compound food in the vivarium, i.e., by a suboptimal diet. This pathology may be exacerbated by elements of the food that are absent in natural food, such as genetically modified (GM) ingredients (GM soybean or corn meal) or contaminants (pesticides, mycotoxins, heavy metals, etc.). Probably, hair growth in the gingival pouches is a protective reaction of the body suppressing the progress of gingival pathology, because the hair bundles are so dense that they prevent food from getting into the pouches and the resultant inevitable inflammation. Hair grows in the parts of the mucosa that, being affected by mechanical factors, acquire the capacity for keratinization.

More recently, Tudisco et al (2010) studied the possible effects on cell metabolism, by determination of several specific enzymes in serum of goats fed conventional or Roundup Ready soybean and in heart, skeletal muscle, liver and kidney of their offspring. Aspartate aminotransferase (AST) and ALT enzyme activity were significantly lower in serum from goats fed GM soybean but enzyme levels were in the normal range. Statistical differences were detected in kid's kidney for GGT and LDH, whereas in the heart and skeletal muscle this result was seen only for LDH. The increase in LDH activity was confirmed by histochemistry. In addition, significant differences between control and treated animals were detected for heart, kidney, muscles and liver LDH isoenzyme distribution, particularly concerning the LDH1, as found previously in the rabbits. Since LDH1 is known to be involved in cell metabolism by favouring the reaction of lactate to pyruvate (Van Hall, 2000), these results could indicate a general increase in cell metabolism.

A summary of experimental studies concerning health of animals fed genetically modified soybean is reported in Table 4.

Animal species	Length of study	Main effects	Reference
BN rats and B10A mice	15 weeks	No immunotoxic activity. No histopathological abnormalities.	Teshima et al. (2000)
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)
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)
Mice	28 days	No adverse effects	Mathesius et al. (2009)
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)
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)
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)
Mice	-	Enlargements in the smooth endoplasmic reticulum of Sertoli cells	Vecchio et al. (2004)
Mice	-	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. GM-fed mice showed mitochondrial and nuclear modifications indicative of reduced metabolic rate	Malatesta et al. (2008a)



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)
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) and (Malatesta et al., 2002b)
Wistar rats	30 days	Significant zymogen-granule depletion	Magaña-Gómez et al. (2008)
Pregnant Rats	2 weeks	Fewer pups born with smaller body mass	Ermakova et al. (2006)
Rabbits	60 days	Significant alteration of kidney ALT, GGT and LDH activity. Significant alteration of heart LDH activity	Tudisco et al. (2006)
Hamsters	-	Growth of hairs inside the pouches of the mouth and the number of hairy mouthed	Baranov et al. (2010)
Kids	60 days	Significant alteration of kidney GGT and LDH activity. Significant alteration of LDH isoenzyme distribution in heart, kidney, muscle and liver	Tudisco et al. (2010)

Table 4. A summary of experimental studies concerning health of animals fed with genetically modified soybean.

## 7. Human/animal safety of glyphosate

Glyphosate is not a genetically modified product but because its use in agriculture is inseparable from the cultivation of herbicide-tolerant GM crops in a particular technology package, its effects on health need to be examined also with that of the glyphosate-resistant GM crops. Although the declared aim of the introduction of glyphosate resistant GM crops was that with these crops the amount of herbicide sprayed on the land should decrease, due to the ever increasing area of cultivation of glyphosate-resistant Roundup Ready GM crops, the use of glyphosate has in fact increased (Benbrook, 2004; 2009). The glyphosate-containing sprays destroy all weeds but the growth of the glyphosate-resistant GM crop is protected regardless of how much glyphosate is sprayed on to the land. To make sure that all weeds are destroyed the use of glyphosate and consequently the glyphosate load of the land has been substantially increasing after the first few years of a slight reduction (Benbrook, 2004; 2009). This has happened despite the ever-increasing number of publications showing that glyphosate has many serious and detrimental effects on the environment and biodiversity (Relyea 2005) with the development of herbicide-resistant weeds (Duke, 2005; Owen & Zelaya 2005; Warwick et al., 2008; Zelaya et al., 2007). There is also an urgent need to consider the potentially seriously damaging effects of this total

herbicide on human/animal health, particularly as it is used in large amounts. Indeed, there are a number of recently published papers that all indicate possible damaging effects of glyphosate on health and reproduction which need to be taken seriously. On previous work the findings of Marc et al. (2005) have confirmed and extended their previous results by showing that the main ingredient of commercial Roundup formulations, glyphosate, in a mM concentration range, particularly when used together with the obligatory polyoxyethylene amine surfactant, inhibited the transcription of one of the enzymes involved in hatching of sea urchin embryos and therefore significantly delayed their hatching. When it is considered that farm workers inhale commercial herbicide sprays in which the active ingredient concentration exceeds by about 25 times of that used in the transcription inhibition studies by Marc et al. (2005), health concerns due to the use of glyphosate must be acute. In another study it was shown that in the oral treatment of Wistar rats with increasing concentrations of the herbicide Glyphosate-Biocarb, a formulation used in many countries such as Brazil, the number of Kupffer cells in hepatic sinusoids increased, followed by large deposition of reticulin fibres and the leakage of hepatic aspartate aminotransferase and alanine aminotransferase into the circulation, indicating hepatic damage in these animals (Benedetti et al., 2004). Successively, Richard et al. (2005) and Benachour et al. (2007) showed that glyphosate, particularly as used together with polyoxyethylene amine surfactant in Roundup Ready formulations, was toxic to human placental JEG3 cells at concentrations lower than that used in agricultural practices. Even at subtoxic concentrations RR was an endocrine disruptor on aromatase activity and its mRNA level as glyphosate interacted with the active site of the purified enzyme. It is possible that the pregnancy problems in agricultural workers using Roundup may be traced back to the exposure to this herbicide (Savitz et al., 2000).

Recently, Gasnier et al. (2009) exposed human liver HepG2 cells, a well-known model to study xenobiotic toxicity, to four different formulations and to glyphosate, which is usually tested alone in chronic *in vivo* regulatory studies. They measured cytotoxicity with three assays (Alamar Blue®, MTT, ToxiLight®), plus genotoxicity (comet assay), anti-estrogenic (on ER<sub>α</sub>, ER<sub>β</sub>) and anti-androgenic effects (on AR) using gene reporter tests. They also checked androgen to estrogen conversion by aromatase activity and mRNA. All parameters were disrupted at sub-agricultural doses with all formulations within 24 h. These effects were more dependent on the formulation than on the glyphosate concentration. First, they observed a human cell endocrine disruption from 0.5ppm on the androgen receptor in MDA-MB453-kb2 cells for the most active formulation (R400), then from 2ppm the transcriptional activities on both estrogen receptors were also inhibited on HepG2. Aromatase transcription and activity were disrupted from 10 ppm. Cytotoxic effects started at 10ppm with Alamar Blue assay (the most sensitive), and DNA damages at 5 ppm. According to the authors, the direct G action is most probably amplified by vesicles formed by adjuvants or detergent-like substances that allow cell penetration, stability, and probably change its bioavailability and thus metabolism. These detergents can also be present in rivers as polluting contaminants. The type of formulation should then be identified precisely in epidemiological studies of G-based herbicides effects. Of course to drive hypotheses on *in vivo* effects, not only dilution in the body, elimination, metabolism, but also bioaccumulation and time-amplified effects should be taken into account. These herbicides mixtures also present endocrine effects on human cells, at doses far below agricultural dilutions and toxic levels on mitochondrial activities and membrane integrity.

All these findings indicate that there is an urgent need to carry out systematic and direct studies, independent of the biotech industry, on the short- and long-term effects on animal (and human) health of exposure to glyphosate and its more effective commercial formulations alone and/or preferably in combination with the appropriate GM crop. With the presently cultivated huge areas of Roundup Ready crops and the anticipated even larger future extensions of this glyphosate-dependent GM crop technology the potential danger for animal/human health needs to be dealt with in advance and not if or when it occurs. If we consider that RR soybeans may in themselves damage reproduction, a combination of the similar, possibly synergistic effects of the GM crop and glyphosate could be a potential disaster waiting to happen.

## 8. Conclusions

The debate on the safety of genetically modified organisms (GMOs) used for food and feed is still very lively throughout the world, more than 15 years after their first commercial release. Huge social, economical, and political issues have been raised. Unfortunately, although some stakeholders claim that a history of safe use of GMOs can be upheld, there are no human or animal epidemiological studies to support such a claim as yet, in particular because of the lack of labeling and traceability in GMO-producing countries. As a matter of fact, 97% of edible GMOs among cultivated GMOs (soy, corn and oilseed rape or canola, excluding cotton) are grown in South and North America, where GMOs are not labeled. All these plants have been modified to tolerate and/or produce one or more pesticides, and contain therefore such residues at various levels. Most are Roundup residues (it is a major herbicide used worldwide and tolerated by about 80% of GMOs).

As stated by the EFSA (2008), several aspects have to be investigated when considering whether or not recombinant DNA from GM plants, or the derived proteins can end up in animal tissues and products. These include (i) the fate of the recombinant DNA and protein during feed processing and ensiling; (ii) the fate of the recombinant DNA and protein in the gastrointestinal tract of animals fed with GM feed; (iii) the potential absorption of the digested pieces of DNA or protein into animal tissues/products and (iv) the potential of biological functionality of absorbed DNA and protein fragments.

The mere detection of recombinant DNA fragments in animal organs and tissues could not justify, by itself, public concerns regarding human consumption of products from farm animals fed transgenic crops. However, the persistence of DNA after dietary exposure is one aspect of risk assessment for novel food. Indeed, as concerns the hypothetical horizontal gene transfer of recombinant DNA from GM crop-derived feeds to animal and human gut microflora, Netherwood et al. (2004), found that a small proportion of feed DNA survives passage through the human upper gastrointestinal tract and a very small proportion of the small intestinal microflora containing transgenic feed. According to the authors, even if this result does not indicate a complete transgenic transfer to the prokaryotes, the survival of transgenic DNA during the passage through the small intestine should be considered in future safety assessments of GM foods. In addition, any alteration in cell metabolism should be taken into account in this field. For instance, the modification in LDH synthesis suggests an increase in cell metabolism. Therefore, possible long-term effects of such an alteration need to be elucidated.

In conclusion, taking into account the potential risks related to GMP impact, further researches are needed in this area, including studies to determine DNA transport or entry

mechanisms/processes across the epithelial layer of the gastro-intestinal tract into the bloodstream, as well as degradation or accumulation of foreign DNA in blood or other organs of animal species. In any case, the traceability of products from animals fed on GMOs is crucial.

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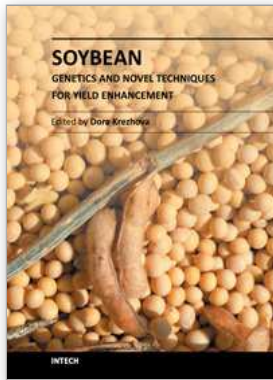
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