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Feeds derived through modern biotechnology: Principle, safety and substantial equivalence

A. Aumaitre
Centre de Rennes, INRA, 35590 Saint-Gilles, France

SUMMARY – Genetically engineered plants, soya-bean, maize, rapeseed, and their by-products grown over 25 million hectares in 1998 in the world are mainly used as foods and feeds for human and farm animals. Principles of this engineering are briefly described as well as the procedures edited in the Official Directives of the EU and used to assess the safety of inserted nucleic acid and expressed proteins. The safety is assessed by the analysis of the genetic construct and on the evaluation of the risk for human, animal and the environment. The substantial equivalence of the products, particularly of grains and seeds has been established according to international rules on the basis of detailed proximate chemical analysis, content in amino acids and fatty acids, vitamins, minerals and trace elements, compared to material issued from isogenic parental lines. The comparison also includes the content in antinutritional factors such as lectins, glucosinolates, antitrypsic factors, solanin, tannins. A significant reduction in the level of mycotoxin fumonisin has been observed on genetically modified maize kernel engineered for resistance to the European corn borer, linked with a decrease in the contamination by *Fusarium* moulds through less damaged ears. Additional evidences on both the safety and the nutritional equivalence have also been provided by the results of chronic experiments conducted *in vivo* on laboratory and farm animals, including dairy cows, chickens and laying hens, and published in international referred scientific literature. On the basis of the present knowledge, feeds derived from genetically modified plants authorised in the European Union can be considered as safe constituents to be incorporated in diets of farm animals.

Key words: GM plants, safety, animal feeds, nutritional equivalence.

RESUME – *"Aliments pour bétail issus des biotechnologies modernes : Principe, sécurité et équivalence substantielle". En 1998, les produits végétaux provenant de 25 millions d'hectares de plantes génétiquement modifiées (grain et còproduits, fourrages) ont été utilisés dans l'alimentation humaine et animale. Les principes de leur obtention sont brièvement décrits ainsi que les dispositions réglementaires appliquées au niveau de l'Union Européenne pour vérifier la sécurité alimentaire des nouvelles plantes et démontrer l'innocuité des gènes introduits et des protéines exprimées, en vue d'autoriser leur diffusion. La sécurité est garantie sur la base de l'analyse de la construction génétique et de ses conséquences pour les plantes et sur la sécurité pour l'animal et l'environnement. L'équivalence en substance des produits et dérivés des plantes est préalablement vérifiée à partir de l'analyse chimique élémentaire mais aussi sur la base des teneurs en acides aminés, en acides gras, vitamines, minéraux et oligoéléments. Les teneurs en facteurs antinutritionnels, lectines, glucosinolates, facteurs antitrypsiques, hémagglutinines, solanine, tanins sont également comparées par rapport à celles observées dans une plante parentale isogénique. Une réduction significative de la teneur en mycotoxine fumonisine des grains de maïs résistants à la pyrale est observée en raison d'une réduction de la contamination de l'épi par le* Fusarium*, qui s'introduit dans les blessures occasionnées par la larve de l'insecte. Des résultats d'expériences d'alimentation chronique à base d'aliments issus de plantes transgéniques, publiés dans la littérature scientifique confirment à la fois leur innocuité et leur équivalence alimentaire pour les animaux. Dans l'état actuel des connaissances, les aliments issus de plantes transgéniques autorisés dans l'Union Européenne ne présentent pas de risques toxicologiques et nutritionnels pour les animaux domestiques et pour leurs produits.*

Mots-clés : *Plantes GM, sécurité, aliment pour bétail, équivalence nutritionnelle.*

Introduction

After intensive laboratory investigations developed since 1985, numerous genetically engineered plants are presently moving to massive market introduction in North America and in Europe (Chesson and Flint, 1999). Data for 1998 indicated that 23 million hectares representing 25 to 45% of the cultivated area of 4 major plants (maize, soya-bean, cotton and rapeseed) consisted in genetically modified (GM) plants destined to produce animal feeds. Between 1998 and 1999, the US production issued from the only glyphosate tolerant soya-bean variety increased to 16 million hectares, driving the percentage of GM soya up to 56% of the total crop.

Production and use of these plants and plant products had been first approved by the Scientific authorities of the US Department of Agriculture (Food and Drug Administration and Environment Protection Agency) by considering that transgenic plants were selected thanks to a safe and accurate methodology used alternatively with other selection methods. The production and spreading of novel foods and novel feeds deriving from genetically modified organisms (GMO) must received the assessment of numerous independent international and national regulatory authorities such as OECD, FAO/WHO, UK, Scandinavian, Dutch, French specialised Commissions and European Union Scientific Committees.

But despite of a final approval at the national and at the European levels for the cultivation and/or he use of several GM plants, only limited attempts have been realised in 1998-1999 in Europe to grow these new plants and a moratorium stopping since June 1999 the authorisation of new constructs is still pending for a period of 2 years. Thus, following the EU Environment Council in June 1999, ministers have committed themselves to a rigorously precautionary approach to authorisation of next GMO applications, pending approval of the revised Directive 90/220 on GMOs.

Opposed to that, products or by products such as soya-bean, rapeseed and their derivatives, and gluten feed issued from GM maize are offered on the European market and are consequently used in foods and feeds for animals. Approval or disapproval of the use of these products derived from GM plants depends mostly on their safety for man, animals and the environment (Directive 90/220 EEC; Federal Register, 1992). Up to 1999, the DG XXIV or "Consumer Policy and Consumer Protection", now Directorate General "Health and Consumer Protection" have been in charge through the Scientific Committee on Plant (SCP) and the Scientific Committee on Animal Nutrition (SCAN) to evaluate the safety of the construct and of the final product on a scientific basis and to deliver advises to the Commission for a decision on approval of the product described in the dossiers. The European Commission has issued a final approval for several plants, particularly for import of seeds and derivatives (maize, rapeseed, soya-bean, cotton) and in a limited number of cases for cultivation (rapeseed, maize).

The aim of the present paper is to introduce and discuss:

(i) The concept and principles of genetic manipulation applied to plants used in animal feeds.

(ii) The procedures used for safety evaluation of feeds derived through modern biotechnology.

(iii) The substantial equivalence in nutritional and antinutritional factors.

On this basis, the feed manufacturers and producers can raise scientific information to be confident with the procedures and to evaluate the safety of the products for the environment and the animals, and as a consequence, to evaluate the safety of the animals products destined to the consumer.

Concepts and principles of genetically modified (GM) plants

Gene cloning in plants has been rather successful in the past few years. By using a gene of agronomic interest engineered in a gene construct, it is possible to introduce resistance to selected pests or to selected herbicides. Marker genes such as antibiotic resistance genes have been intensively used as efficient tools in the selection of the transformants (Fig. 1).

Thus this foreign DNA generally codes for new enzymatic proteins with different biological activities. There is also a possible impact of the new materials in the use of GMOs as food for humans, feeds for animals, on human health and the environment. As a consequence, a comprehensive assessment of the implication of antimicrobial resistance and the presence of foreign DNA and proteins has been required.

Use of antibiotic resistance marker genes

Numerous marker genes have been routinely used in the selection of plant transformants despite

they do not serve any function in the final crop plant. Neomycin phosphotransferase (npt II) is widely used as a selectable marker (Table 1). It confers kanamycin or neomycin resistance, two antibiotics not absorbable, and less and less used in human and animal therapy. Other less common plant transformants include streptomycin resistance and gentamycin resistance genes (Kärenlempi, pers. comm.).

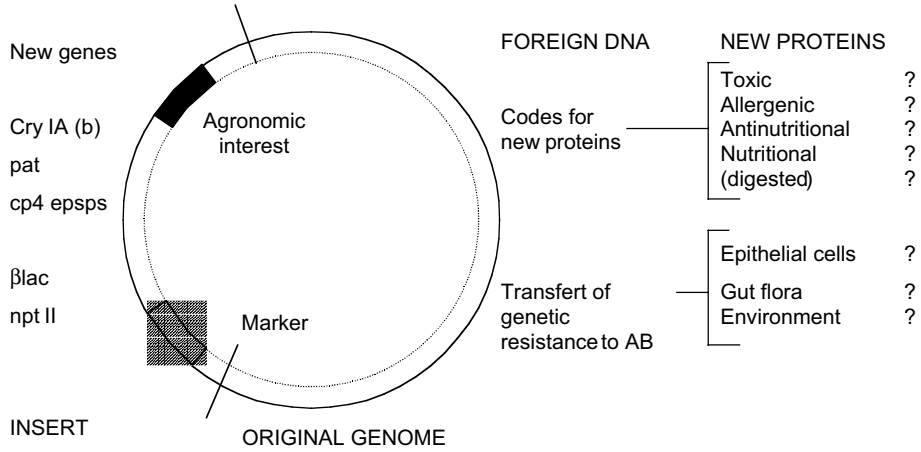

Fig. 1. Principe and potential hazard of genetically modified plants for farm animals.

Table 1. Marker genes of antibiotic resistance used in the design of transgenic plants in the dossiers submitted to the FDA/EPA for approval† (US/FDA, 1998)

Plant	No. of dossiers	Used for selection	Under controls of bacterial promotors
Maize	9	–	3 npt II 5 bla 1 cat
Rapeseed	5	3 npt II	–
Cotton	4	3 npt II	2 aad
Potato	2	2 npt II	1 aad
Soya bean	2	1 npt II	1 bla

†npt II: neomycin; bla: β lactamase; cat: chloramphenicol; aad: spectinamycin-streptomycin.

Another use of antibiotic resistance gene markers is for selection of bacterial transformants. In this case, the vector used in the genetic construct contains bla genes (or β lactamase) conferring resistance to ampicillin. It has been intensively used for generating maize, oilseed rape, beet, potato and alfalfa. Other antibiotic resistance genes used less frequently in the constructs include chloramphenicol, kanamycin, streptomycin-spectinomycin and tetracycline resistance genes (Table 1).

Expressed proteins

A few examples will be given to characterise the expression of special proteins in the GM plants already accepted for dissemination.

The cp4 epsps which confers the resistance to glyphosate is expressed in very low levels in GM soya-bean, accounting for between 0.019 and 0.040 per cent of the soya-bean seed by weight, as determined with validated ELISA assays with seeds derived from 10 field tests conducted in 1992. Cp4 epsps accounted for no more than 0.1 per cent of the total protein in either soya-bean seed or processed fractions prepared from these soya-beans. In addition, cp4 epsps activity was not detected in toasted soya-beans meal fractions, protein isolate or protein concentrate prepared from these soya-beans due to inactivation upon heat processing (Fuchs *et al.*, 1996).

The Cry IA (b) which confer the resistance to the European corn borer is also present in very low amounts in roots, pith and kernels. A maximum amount has been found in leaves at anthesis but the concentration falls gradually up to the senescence of the plant. Additional data on the behaviour of these expressed proteins concerns a full breakdown during the fermentation of the silage (Table 2). These indications are of great practical importance to assess the safety of the whole plant, which is generally destined to herbivorous animals after storage by silage process.

Table 2. Cry IA (b) levels in various tissues, whole/plant and silage in a hybrid insect resistant Bt maize (Fearing *et al.*, 1997)

	Tissue leaves† (senescence)	Roots	Pith	Kernel	Whole plant	Silage (whole plant)
ng Cry IA (b)/g fresh weight	200	<8	<8	<5	50	–
ng Cry IA (b)/g DM	–	–	–	–	120 ±42	0 –

†Up to 3000 ng/g fresh weight at anthesis.

Safety of inserted and expressed products in GM plants

Safety of nucleic acids and proteins

In the assessment of the safety of material issued from genetic transformation and ingested by farm animals, the initial hypothesis of the possible breakdown in the digestive tract should be examined (Fig. 2).

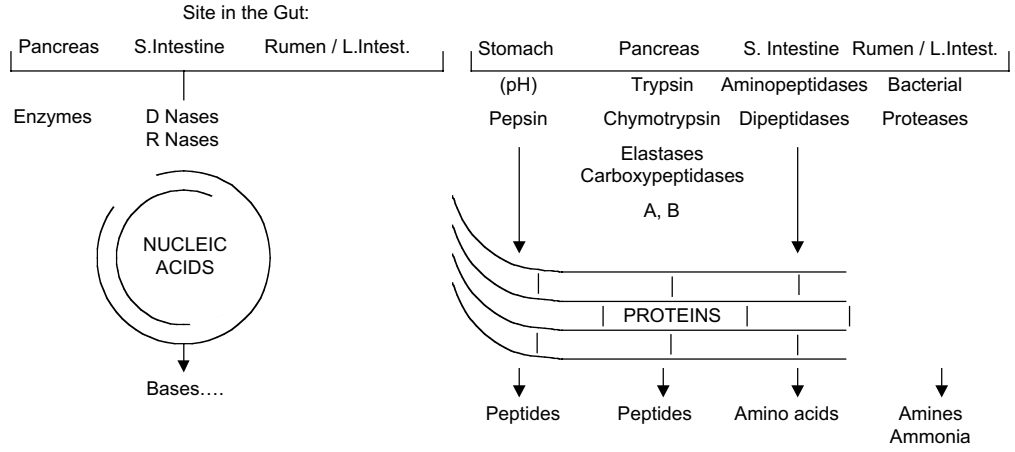

Fig. 2. Breakdown of inserted genes (nucleic acids) and expressed protein in the digestive tract of animals fed genetically modified plants.

In general, the amount of marker gene (DNA) in feed is negligible in comparison with the quantity

and variety of DNA present in the digestive tract resulting from feed residues, bacteria, and cell desquamation. The presence of large amounts of DNases and RNases in the pancreatic secretions and in the bacterial flora of the rumen and of the intestine leads to the hypothesis of a potential breakdown of nucleic acids as demonstrated since a long time in the rumen of steers (Fig. 3). The breakdown of expressed proteins in the digestive tract is even more obvious when in monogastrics as in ruminant species, numerous proteases are present and active in the hydrolysis of ingested proteins (Fig. 2). Further data on the amino acid sequence of the proteins can also be useful indicators of their degradability by most active pancreatic enzymes.

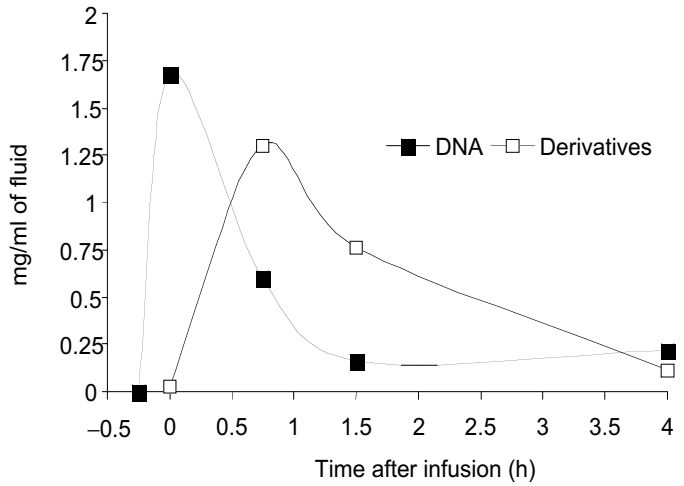

Fig. 3. Disappearance of DNA and its derivatives after infusion *in vivo* in the rumen of steers. Adapted from Mc Allan and Smith (1973).

The initial assessment of proteins *per se*, expressed as a result of genetic modification has first been focussed on *in vitro* digestibility. Proteins that are digestible and have no record of toxicity in higher vertebrates do not require further toxicological testing. In general, proteins introduced by genetic modification will not pose problems after their ingestion because they are microconstituents in the feed. In case where the safety of proteins introduced by genetic modification cannot be established by assessment of the digestibility, function or toxicity of the protein, carefully designed toxicological studies on the protein may be needed.

As an example, the toxicological profile of Phosphinothricin Acetyltransferase (pat) protein involved in the resistance to the herbicide glufosinate has clearly been established by *in vitro* studies of the protein generated by transformed *E. coli*. The acute oral toxicity test of the bacterially derived pat protein showed no tests substance related death or degraded performance up to 2500 mg/kg of body weight (Federal Register, 1997). Similarly, the potential toxicity test established for npt II protein administered by gavage of mice demonstrated no deleterious effect up to the dose of 5000 mg/kg body weight (Table 3). Upon ingestion, npt II apparently does not cause any detectable harm (Nap *et al.*, 1992) because it is inactivated and degraded by the acidic environment and digestive enzymes to a level that will cause no concern (Fuchs *et al.*, 1993). Similar tests on the preliminary analysis of the results of a 91 days feeding trial of transgenic Bt tomatoes in rats did not reveal any sign of adverse effect of the bacillus insecticidal crystal protein Cry IA (b) expressed in transgenic tomatoes (Noteborn *et al.*, 1994).

Test on the potential allergenicity

All the expressed proteins have been tested for their potential allergenic effects. The test requires purification or production of the pure protein. The most recommended test consists in a comparison of their biochemical properties to the properties of commonly allergenic proteins (Table 4). In general, cp4 epsps, Bt and pat proteins only share a similar molecular weight but no other characteristics, in

particular, they are not glycosylated. In addition, an intensive use of plant seeds and their derivatives in animal feeds during the past 5 years in North America confirms this hypothesis on non allergenic properties of selected expressed proteins for target animals.

Table 3. Summary of results of the potential toxicity test of the npt II (neomycin phosphotransferase) administered by gavage in mice

Test group (mg/kg BW)†	Body weight (g)		Food consumption (g/d)	
	Male	Female	Male	Female
0	28.4	22.9	4.9	9.4
100	28.4	22.2	4.7	5.8
1000	28.3	22.0	5.4	7.5
5000	28.5	22.0	5.2	6.1

†mg/kg body weight. Adapted from Fuchs *et al.* (1993).

Table 4. Comparison of the biochemical characteristics of cp4 epsps involved in the protection of plants against pest or herbicides

	Allergenic proteins	Cp4 epsps†	Bt††	Pat††
Molecular weight 10-70 Kd	+	+	+	+
Prevalent protein in feed	+	–	–	–
Stability to digestion	+	–	–	–
Stability to treatments (temp)	+	–	–	–
Glycosylation	+	–	–	–
Similar to allergens	(+)	–	–	–

†Adapted from OECD (1996).
††Adapted from Taylor *et al.* (1987).

Potential risk in gene transfer

The huge amount of DNA that passes the digestive system daily indicates that DNA in itself is not intrinsically toxic to animals. Most DNA is efficiently degraded in the intestinal tract as non functional gene are assumed to remain present (Nap *et al.*, 1992). But because the transfer of antibiotic resistance to clinically important bacteria has been observed, it is important to consider this risk in the case of GM plants bearing genes of antibiotic resistance. A panel of independent scientists raised by the US Department of Health and Human Services, and the Food and Drug Administration (US/FDA, 1998) has examined 3 hypothesis for the safety assessment of the insertion of antibiotic resistance genes, to the epithelial cells, to gut microorganisms, and to the environment.

The transfer of antibiotic marker genes to gut epithelial cells is considered to be unlikely because the DNA is degraded by the nucleases and epithelial cells are short-lived (7 d) and would be replaced by new cells.

The transfer to gut microorganisms: in the digestive tract, approximately 10^{14} bacteria are present, 10^{12} of them do not bear kanamycin resistant and no data are available on the possible transfer. In addition, no known mechanism for the direct transfer of plant genomic DNA to microorganisms is available. Besides to the degradation of DNA, there is an absence of homologous end for efficient transfer.

The transfer to the microorganisms of the environment: the DNA issued from plant debris would be unavailable for transfer after being degraded by nucleases. In addition, the transfer from bacteria to

bacteria accounts for the wide dissemination of certain antibiotic resistance markers in soil bacteria.

Requirements of the European Union on feed safety aspects

Because of a lack of information in the Council Directive 90/220 EEC concerning the release of the GMO into the environment in its consequences on the safety of animal feeds, the SCP has recently proposed a guidance document to assess feed safety aspects (Fig. 4).

Product description and intended use (plant, plant parts)
Origin of genes products used for safety assessment (expressed proteins)
Target animals (feeding experiments)
Degradation in the digestive tract (*in vivo*); (+ *in vitro*)
Use of intact plants or by-products
Substantial equivalence (// isogenic; ; 2 seasons; /= geographical location)

Fig. 4. Requirements of the EU on feed safety aspects of products and by-products issued from GM plants. Adapted from SCP/GMO (1999).

The dossiers submitted by the reporters should contain information related to the sequence inserted/deleted, the expression of the insert and information on the risk assessment related to the genes inserted. But the main recommendation regarding animals (and man) are related with toxicology and residues. Residues of plant protection products such as pesticides and herbicides are first concerned, including the active molecule and its derivatives. The most important precaution is devoted to the safety of foods and feeds. The recommendations include the product description and intended use of the plant and the plant parts, the origin of gene products used for safety assessment.

Additional recommendations concern the requirements for feeding experiments on target animals as well as *in vivo* tests for the evidence of the degradation of the inserted and expressed products in the digestive tract. Feeding studies with the GM plants or their by-products should be performed in addition with studies on the degradation of the expressed products (proteins) *in vivo* in the digestive tract on target animals. *In vitro* simulation of gastric and intestinal digestion are only considered as supplementary evidences to *in vivo* results. The case of intact plants, plant parts and by-products specially where a concentration of introduced gene products is expected is also required. Evidence of *in vivo* degradation of the introduced gene product should be given separately for monogastric and ruminant species. In addition, in case of a potential concentration of introduced gene products, safety and substantial equivalence are also required. As an example, the extraction of oil in oil seeds, the extraction of starch in maize and potatoes or the extraction of proteins fractions can lead to a concentration of the expressed protein in the by product used as feed. But the final recommendations are relative to the substantial and nutritional equivalence, both tested by appropriate chemical analysis and experimental tests on target animals during their productive life, respectively.

Substantial (and nutritional) equivalence

Substantial equivalence

According to the recommendations of the SCP (SCP/GMO, 1999), substantial equivalence particularly well demonstrated in first generation crops (Chesson and Flint, 1999) must be assessed by the determination of proximate chemical composition for major nutrients including amino acids and fatty acids, but also vitamins and trace elements. Additional determinations of antinutritional factors are specially required in selected feedstuffs such as soya-bean (antitrypsic factors, isoflavones), potatoes (solanine, chaconine), cotton (gossypol), and rapeseed (glucosinolates). Such data are absolutely required in the dossiers supplied to the National and European Commissions but they are only published in a very limited number of cases such as for soya-beans and the corresponding meals (Table 5). It is interesting to mention that both lectins and trypsin inhibitors are equally present in the seeds, but destroyed by the technological process for oil extraction. As a consequence, potential risks for health and performance of animals resulting in the use of GM plants and by-products are minimised for plants recommended by the Official Scientific Organisations in charge of delivering an

opinion on the safety of the products.

Table 5. Comparative substantial equivalence in parental and genetically modified soya bean and soya bean oil meal (glyphosate tolerant). Adapted from Padgette *et al.* (1996)

	Seed		Oil meal	
	Control	GMO	Control	GMO
Chemical analysis				
Protein	41.6	41.4	54.4	54.4
Lysine	2.61	2.56	–	–
Fat	15.5 a	16.3 b	2.3	2.0
Oleic acid (% of f. acids)	52.5	52.3	–	–
Phytates	–	–	1.76	1.81
Stachyose	–	–	5.7	6.0
Raffinose	–	–	0.96	1.11
Lectins				
U.H/mg DM	1.2	1.0	<0.01	<0.01
Trypsin inhibitors				
IU/mg DM	43.0	45.0	3.4	3.3
Isoflavones				
Genistein µg/g DM	833	830	938	976

[a,b]Significant differences.

Since the primary use (approximately 97 per cent) of soya-beans is as a supplement for animal feed, selected animal wholesomeness studies demonstrate the substantial equivalence of the soya beans derived from the glyphosate-tolerant line as compared with the isogenic variety. Based on extensive compositional analysis performed, no difference have been found either on the content of major nutrients, nor for potential antinutritional factors such as α galactosides, lectins, antitryptic factors (Table 5).

Nutritional equivalence

This expectation was confirmed in wholesomeness studies performed with dairy cow, chicken and catfish (Tables 6 and 7). These studies were designed as wholesomeness studies to measure the ability to support growth and well being of the animals. There were no statistically significant differences in feed conversion in any of these studies between the glyphosate tolerant soya beans and the parental variety. Despite the fact that the experiments were not designed to be toxicological studies, results were complementary *in vivo* indicators of the absence of ANFs and the product free of any additional deleterious product generated by the presence of the inserted gene. A few complementary data concerning the safety of GM rapeseed are also available. Seeds of substantial equivalence including for their content in glucosinolates were tested for 2 weeks at a 30 per cent level in a complete diet for growing rabbits (Table 8). The seeds from the transgenic entry sustained similar performance and apparent digestibility of nutrients compared to the control group based on isogenic seeds. Additional evidences have been recently supplied on the absence of detrimental effect of an expressed insecticidal bean α amylase inhibitor transferred in peas on the performance and on the digestibility of dietary nitrogen in rats (Pusztai *et al.*, 1999). More promising advantages of products deriving from GM plants have been recently discovered in case of Bt resistant maize which generally contains lower levels of mycotoxin Fumonisin. The absence of damage caused by army worm and maize ear worm on the cob explained a limitation in the contamination of the grain by *Fusarium* ear rot (Munkvold and Hellmich, 1999).

Testing of whole feeds in animals is not generally recommended in the US legislation for feeds derived as a result of genetic modification of food crops and will, in general, be required only for those which cannot be shown substantially equivalent to their traditional counterparts. Animal feeding

studies in general are considered to be not sensitive enough to detect unintended effects as a result of gene modification. On the contrary, animal studies are considered necessary in Europe as an additional test for safety assessment. The concept of wholesomeness of the feed needs to be applied. It demands well designed animal studies performed during a significant duration where concern related to nutritional imbalance is addressed. Nutritional imbalance may mask toxic effects. Consideration could be given to the testing of extracts rather than whole feed. The results from such studies may give clues as to what might constitute a toxicological concern. The testing of specific components of a novel feed may also present an option for addressing additional safety testing.

Table 6. Effect of GM soya bean on production traits of the dairy cow fed 10% of cracked soya bean during a four week-test. Adapted from Hammond *et al.* (1996)

	Control	GM (glyphosate tolerant)
Average feed intake (kg DM/d)	24.4	24.7
Apparent digestibility of DM (%)	69.0	68.6
Milk production (kg/d)	34.9	36.2
Milk protein (%)	3.28	3.23
Milk fat (%)	3.37	3.59

Table 7. Nutritional equivalence of GM soya bean on production traits and quality of animal products in chickens and catfish. Adapted from Hammond *et al.* (1996)

	Chicken (6 weeks)		Catfish (10 weeks)	
	Control	GM	Control	GM
Percent of dietary				
Soya bean meal	32.8/26.6	33.8/26.6	45	47
Performance (100 for control)				
Weight gain	100	98	100	96.5
Feed intake	100	99	100	99
Survival rate	100	101	100	98
Weight of breast muscle	100	97	–	–
Body protein percentage	–	–	100	99

Provisional conclusions

The presence of GM plants and their derivatives on the European market of feeds for animals is obvious since some years whatever consumer's attitude or preference and the requirements for labelling. Because the old continent is only 30% self sufficient for high protein feeds, there are no easy solutions to avoid that these ingredients could be mixed with conventional products and enter the feed chain. Nevertheless, all these products derived from plants the spreading of which has been allowed by Official Committees of the US, Canada and Europe on the basis of a minimum number of information required by the Council Directive 90/220 EEC and that of the Federal Register (1992). It is also clear that new progress is in the pipeline and that one can expect new plants generated by conventional and/or genetic manipulation procedures. Information have been circulated on the possible availability in the near future of aflatoxin resistant maize, sweet potatoes with a high content in protein, rice rich in vitamin A and available iron, potatoes having a high content in amylopectin and possibly cereals with a higher proportion of essential amino acids. A selective excision of antibiotic marker genes is programmed by numerous Companies. In any cases, new plants derived from GM will require a full dossier on both sides of the Atlantic, destined to Scientific Commissions of

independent experts for approval of their use for cultivation and food and feed production. Using data of chemical analysis, substantial equivalence of products must be established by comparison with a preferably isogenic variety with samples grown at least for 2 seasons in different environments. As a final request, the environmental aspects including impact on non-target organisms, non modified crops and wild plants are required. When the substantial equivalence cannot be assessed, a case by case study is an additional request forecasted by the recommendations. National and International Scientific Committees are in charge to examine the content of the dossiers, to assess that the requested information are available and deliver a general conclusion recommending or not the product after the safety has been fully assessed for human and/or for the animal use.

Table 8. Nutritional equivalence of control and GM rapeseed tested on growing rabbits. Adapted from Maertens *et al.* (1996)

Cultivar	Control	GM MS8 x RF3[†]
Chemical composition (% of DM)		
Crude protein	26.3	26.2
Fat	44.3	41.4
NDF	40.1	40.2
ADF	25.5	25.6
Nutritional equivalence[††]		
Feed intake (g/d)	148	157
Weight gain (g/d)	12	14
Digestibility (%)		
Nitrogen	78.7	76.3
Energy	81.4	78.7

[†]Male sterile x restored fertility from PGS, Belgium.
[††]Seeds were tested at a level of 30% of inclusion in the diet.

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