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Substantial Equivalence of Antinutrients and Inherent Plant Toxins in Genetically Modified Novel Foods

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Abstract—For a safety evaluation of foodstuff derived from genetically modified crops, the concept of the substantial equivalence of modified organisms with their parental lines is used following an environmental safety evaluation. To assess the potential pleiotropic effect of genetic modifications on constituents of modified crops data from US and EC documents were investigated with regard to inherent plant toxins and antinutrients. Analysed were documents of rape (glucosinolates, phytate), maize (phytate), tomato (tomatine, solanine, chaconine, lectins, oxalate), potato (solanine, chaconine, protease-inhibitors, phenols) and soybean (protease-inhibitors, lectins, isoflavones, phytate). In several documents used for notifications no declarations even on essential inherent plant toxins and antinutrients could be found, for instance data on phytate in modified maize were provided only in one of four documents. Significant variations in the contents of these compounds in parental and modified plants especially due to environmental influences were observed: drought stress, for example, was made responsible for significantly increased glucosinolate levels of up to 72.6 μmol/g meal in modified and parental rape plants in field trials compared to recommended standard concentrations of less than 30 μmol/g. Taking into account these wide natural variations generally the concentrations of inherent plant toxins and antinutrients in modified products were in the range of the concentrations in parental organisms. The results presented indicate that the concept of the substantial equivalence is useful for the risk assessment of genetically modified organisms (GMOs) used for novel foods but possible environmental influences on constituents of modified crops need more attention. Consistent guidelines, specifying data of relevant compounds which have to be provided for notification documents of specific organisms have to be established. Because of the importance of inherent plant toxins and antinutrients on nutritional safety, also coherent databases of standard parental lines and clear criteria for mandatory declarations are necessary.

Keywords: GMO; novel food; substantial equivalence; inherent plant toxins and antinutrients.

Abbreviations: GMO = genetically modified organisms; GNA = snowdrop bulb lectin; GTC = glufosinate tolerant corns, HU = haemagglutinating units; TIU = trypsin-inhibitor units.

INTRODUCTION

Genetically modified food is becoming an increasing part of the common food supply. Rigorous specifications are necessary to ensure the safety of these products for human health and for the environment. Since 1997, the Novel Food regulation (258/97) regulates the introduction of novel food to the European market following an environmental risk assessment of all genetically modified organisms (GMOs), according to directive 90/220/EEC. In the US the Food and Drug Administration controls the food and feed safety of GMOs by the guidance "Foods Derived From New Plant Varieties".

In the EC, all GMOs that can still reproduce or processed foodstuff from GMOs which are no longer substantial equivalent to their parental organism need an explicit consent of the European member states for marketing. In the case of demonstrated substantial equivalence of key toxic or allergenic compounds, key nutrients and possible inherent plant toxins and antinutrients and a risk assessment of the genetic modification, the modified product can be placed on the European market with a notification only (EC, 1997). Therefore, the assessment of the substantial equivalent is not only

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important for the risk assessment but also decides on regulatory decisions. In the determination of substantial equivalence clearly not only a food compositional analysis but also an assessment of all possible consequences such as agronomic/phenotypic characteristics are an important element (as discussed in the FAO/WHO report Food and Nutrition Paper 61, Rome 1996).

Special attention in the analysis of substantial equivalence has to be focused on inherent toxic and antinutritive constituents, since genetic modification could affect the expression of gene products not addressed by the genetic modification (unintentional pleiotropic effects) and thereby alter the content of constituents (Koschatzky and Massfeller, 1994).

The definition of plant inherent toxicants and antinutrients is still not entirely harmonized. Usually antinutrients are understood as substances that inhibit or block important pathways in the metabolism, especially the digestion. Antinutrients reduce the maximum utilization of nutrients (especially proteins, vitamins or minerals), and as a consequence they obstruct an optimal exploitation of the nutrients present in a food and decrease its nutritive value (Watzl and Leitzmann, 1995). Only substances with primary effects on the availability of nutrients are considered in this paper, not compounds with only toxicological qualities. However, many antinutrients may also be toxic beyond a certain dose, for example oxalate or cyanogenic acid, and the subjects of this study are inherent plant toxins and antinutrients (Akpanyung *et al*., 1995; Isong and Essien, 1996).

Most of the deleterious effects of antinutrients are caused by raw plant material. Most of the antinutritive substances become ineffective by simple measures such as heating, soaking, germination or autoclaving. Recently, data about positive effects of inherent plant toxins and antinutritive substances have been also published, for instance, anticancerogene and antibactericidal qualities were found (Watzl and Leitzmann, 1995). A list of the most frequent and important classes of these constituents, their occurrence and their nutritional effects is given in Table 1.

The expression of constituents of crops such as inherent plant toxins and antinutrients, and thereby their concentrations in a genetically modified plant, can eventually be influenced by pleiotropic effects (http://www.crop.cri.nz/psp/articles.htm). Such effects can occur when integration of the genetic material into the genome leads to non-predictable phenotypic effects, one singular genetic transfer can cause multiple changes in characters. Effects could be increased synthesis activity of the naturally occurring biochemical metabolism pathways, augmented synthesis caused by increased gene activation, decreased synthesis of catabolism enzymes, or reduced decomposition (Koschatzky and Massfeller, 1994). Regulatory elements in the plant DNA can influence the expression of the inserted genes and random insertion events may

Table 1. Classes of the most frequent inherent plant toxins and antinutrients

	Occurrence	Effect
Cyanogenetic glycosides	Maniok, cassava, yams, sweet potato, fruit (stones), millet, phaseolus lunatus, limabean,	Blocking of cell breathing, gastrointestinal symptoms. Influence on carbohydrates and Ca transport. At high intake doses iodine deficiency
Glucosinolates (goitrogen): sinapsin, sinigrin, progoitrin, arachidosid	Cruciferae, esp. in seeds: rape, mustard seeds, radish, cabbage, kale, peanut, soybean, onion, cassava	Strumatic effects (forming of goitre): thyroid gland increase, thyroxin synthesis ↓, metabolism impairments, iodine absorption ↓, protein digestion ↓
Glykoalkaloids (solanine and tomatine)	potato, tomato (*Solanaceae*), unripe fruit	Inhibition of cholinesterase; gastrointestinal symptoms, haemolysis, inflammation of kidney
Gossypol	Cottonseeds	Binds metals, iron absorption ↓, inhibitor of enzymes
Lectins (phytohaemagglutinins)	*Fabaceae*, cereals, soybean, beans	Inflammation and damage of the intestinal epithels, ↓ resorption of nutrients and N retention (→inhibition of protein synthesis), ↓ enzyme activity, ↓ B12 and lipid-resorption
Oxalate	Spinach, celeriac, beetroot, rhubarb, Amaranth, silver beet, tomato	Ca-oxalate crystals, insoluble salts of Ca, Fe or Zn (not resorbable) → Ca metabolism impaired
Phenols (flavonoids, isoflavone, chlorogen acid)	Vegetables, fruit, vine, cereals, soybean, potato, tea, coffee, plant oils	Destruction or inhibition of thiamine, metal-complexes, availability of trace elements ↓, oestrogen effects, hypocholesterolaemic activities
Phytate	All plant seeds, cereals, *Fabaceae*	Complexes: bioavailability of Ca, Mg, Fe, Zn, Cu, Mn ↓, utilisation of protein and starch ↓ (↓ activity of proteolytic and amylolytic enzymes)
Protease-inhibitors	*Fabaceae* seeds, peanut, cereals, rice, maize, batate, potato, apple	Inhibition of trypsin and chymotrypsin, carboxypeptidases and pancreaselastase → ↓ digestion of protein
Saponin	*Fabaceae*, spinach, asparagus, sugar beet, soybean, tea, peanut	Complexes with proteins and lipoides (e.g. cholesterol), haemolytic, gastroenteritis, most saponins harmless
Tannins	Widespread: all fruits, tea, coffee, *Vicia faba*	Inhibition of pancreatic enzymes, cobalamin resorption ↓, thiamine utilization ↓, availability of protein and iron ↓

Table 2. Exemplary contents of glucosinolates (μmol/g seed or meal) in modified rape

New hybridization system (Plant Genetic Systems (1996)) μmol glucosinolates/g

Location	Experiment 1				Experiment 2				Experiment 3			
	Seed[1]		Meal[2]		Seed[1]		Meal[3]		Seed[1]		Meal[3]	
	modif.	control	modif.	control	modif.	control	modif.	control	modif.	control	modif.	control
Belgium	25.03–26.76	25.19/20.26			25.16–27.99	26.75/22.35	42.94–46.83	44.81/36.59	21.79–23.95	21.93–23.04	38.50–41.43	36.58–40.00
France	20.88–23.08	23.08/18.37			22.11–27.68	22.01/22.04	38.53–46.68	38.07/36.95				
UK	16.14–17.77	17.17/15.38			37.89–41.59	41.13/35.03	66.56–72.62	71.19/61.60	20.25–20.85	19.08–20.84	35.00–36.12	33.21–35.24
Canada			26.61–36.63	30.53/22.44			22.58–36.73[2]	32.49/7.26[2]				

[1]Alkenyls + indols/g seed. [2]Alkenyls/g oilfree meal. [3]Alkenyls + indols/g meal.Control means untransformed/local check or range of different control cultivars.

disrupt or modify the expression of existing genes in the recipient plant. The most common events could be gene inactivation or silencing, but gene activation and gene fusion are theoretically also possible. It is the possibility of gene activation which raises the most concern for food safety, especially for genes encoding enzymes in pathways toward the production of deleterious secondary plant compounds (Lang, 1979).

In the case of a possible production of deleterious substances, both the activation of shut-down metabolism pathways and an impaired expression of enzymes which inactivate noxious substances belonging to the plant could occur by genetic modifications (Koschatzky and Massfeller, 1994). Therefore, aspects of pleiotropic effects have to be taken seriously in the assessment of the substantial equivalence. As inherent plant toxins and antinutrients are important constituents in the assessment of the substantial equivalence of key nutrients, the expression of these compounds has been compared in documents of genetically modified crops. The present analysis therefore focuses on possible effects of genetic interventions on inherent plant toxins and antinutritive constituents, and does not analyse safety of modified crops or foods. This class of compounds represents only a subset of the compounds which would be needed for a comprehensive compositional analysis for a food safety assessment.

MATERIAL AND METHODS

For a comparative analysis of the content of inherent plant toxins and antinutrients in genetically modified and parental plants, we evaluated data of these components in documents of genetically modified crops. Some of these crops may be used as food or feedstuff after adequate future approvals. Following common scientific knowledge on inherent plant toxins and antinutrients in plants (Belitz and Grosch, 1992; Füllgraff, 1989; Lang, 1979; Teuscher, 1994) the following consituents have been analysed in specific genetically modified crops: rape plants and canola for glucosinolates and phytate; maize for phytate; tomatoes for tomatine; solanine; chaconine; lectins and oxalate; potatoes for glykoalkaloids; protease-inhibitors and phenols; and soybeans for protease-inhibitors, lectins, isoflavones and phytate.

Data came from non-confidential parts of documents for notifications according to the European Directive 90/220/EEC, Novel Food regulation, product clearance according to the American USDA/FDA or other scientific literature.

Information on these documents is widely available publicly on internet servers or registers of national competent authorities: http://www.aphis.usda.gov/biotech/; http://vm.cfsan.fda.gov/~lrd/biotechm.html; http://biosafety.ihe.be/; http://www.maff.gov.uk/food/foodnov.htm.

RESULTS

It was found that in most genetically modified organisms the levels of inherent plant toxins and antinutrients were within the range of the non-transformed parental organisms. In several documents no or only incomplete data on these compounds could be found. Until now, there are no specific regulations that specify which inherent plant toxins and antinutrients have to be declared and tested in which plant, and consequently there is also no consistency according to which companies can proceed for their analysis. Some documents provided no data at all about these constituents or conclude that some inherent plant toxins and antinutrients are not relevant, for example saponins in tomato (Zeneca, 1996) or phenolic compounds and couramins in potato (Avebe, 1996). Only in one of four documents of modified maize plants was phytate analysed. In the investigated dossier of modified tomato, many antinutrients were tested, such as glykoalkaloids and lectins, but no data were provided about oxalate (Zeneca, 1996). With regard to modified soybeans, the analysis of lectins and isoflavones are controverse; in one dossier their levels are indeed determined (Padgette *et al.*, 1996), in another they are not (Du Pont, 1996). Also, the determination of inherent plant toxins and antinutrients in potato are not coherent, data on chlorogenic acid are provided in one document (Amylogene, 1996), but not in other (Avebe, 1996); the same problem was found with trypsin inhibitors.

In one dossier on modified rape plants (Plant Genetic Systems, 1996) the values of glucosinolates in the modified plant were significantly different (higher) than in the non-transformed plant, and in some cases significant differences between the glucosinate content of one modified line and its non-transgenic counterpart were observed. In a dossier of modified potatoes (Amylogene, 1996), the values of glykoalkaloids were significantly lower than in the control line.

Rape

The genetic modification of oilseed rap aims mostly at a new fatty acid pattern or at a resistance against herbicides and pests. As important inherent plant toxins and antinutrients, oilseed rape contains glucosinolates, and also phytic acid in the oil-free meal (Plant Genetic Systems, 1996). Erucic acid would have to be analysed in an assessment of toxicology of the oil. Several documents of notification dossiers of genetically modified rape plants (AgrEvo, 1992, 1997; Plant Genetic Systems, 1996) have been analysed for data on inherent plant toxins and antinutrients.

In one document of genetically modified rape plants (Plant Genetic Systems, 1996), in some cases significant differences between the glucosinate content of one modified line and its non-transgenic counterpart were observed. It was noticed that the glucosinolate content varied more between the different locations than between the different transgenic and non-transgenic entries in a given location. These variations were discussed, suggesting it is generally accepted that commercial food derived from plants exhibits considerable variability in its composition and that this variability is more the result of the interaction of the genotype with the environment, rather than the result of the insertion of specific genes into the plant genome. In this respect, it was furthermore suggested that normal agricultural breeding practices will ensure that the glucosinolate level of the parental lines and the restored hybrid products is according to standards for canola seed (< 20 μmol alkenyls/g oil-free meal). In this document it is affirmed that the collected data of the genetic modified lines fit within the range established for oilseed rape (Plant Genetic Systems, 1996). However, it is also stated that the biochemical analysis data of transformed and non-transformed seeds show that, for instance in releases in Belgium the glucosinolates per gram of seed and per gram of meal are significantly higher than the control (Plant Genetic Systems, 1996).

Quality standards for oilseed rape meal allow not more than 30 μmol of total glucosinolates (total of gluconapin, glucobrassicanapin, progoitrin and napoleiferin) per gram of defatted meal. Analyses of different entries, modified lines and local controls grown in different regions provide variable results.

An overall consideration of all data provided in the dossier on glucosinolates in transgenic rape plants showed wide variations with levels for meal between 8 and 73 μmol glucosinolates/g oil-free meal, and for seeds between 11 and 42 μmol glucosinolates/g oil-free seed (see Table 2).

Some striking results have to be considered in detail: in several sites the contents of glucosinolates of the modified lines, but sometimes also of the untransformed plants, were higher than 30 μmol/g meal (see Table 2). In one seed, quality analysis experiment values were between 66.56 and 72.62 μmol glucosinolates/g *meal* for the transformed lines, which is far above the quality standard of 30 μmol/g, and also high levels were found in the seeds of 37.89–41.59 μmol glucosinolates/g *seed* (see Table 2). The similar increase of the glucosinolate level of all entries is said to be caused by drought stress (Plant Genetic Systems, 1996).

Although in some cases statistically significant differences in seed quality data were noted between the lines, however, documents claim that the genetic modified lines fit within the range established for oilseed rape (Plant Genetic Systems, 1996).

Canola is a trademark term that is presently defined as seed, oil and meal from *Brassica napus* and *B. rapa* plants that contain no more than 30 μmol of aliphatic glucosinolates/g of oil-free, moisture-free meal (AgrEvo, 1997).

Analysis of two notifications of glufosinate tolerant canola crops which have been released in 1997 (HCN28) and 1995 (HCN92) (AgrEvo, 1992, 1997), show without exception that glucosinolate levels are less than 20 μmol/g. HCN28 meal consistently had glucosinolate levels of 12.4 μmol/g or less. HCN92 had levels of 5.0–8.0 μmol/g (see Table 3).

Quality analysis of HCN28 seed and of HCN92 confirmed that the levels of total glucosinolate compounds were below the mandatory concentrations established for commercial canola varieties (AgrEvo, 1992, 1997); thus documents conclude that HCN28 does not present a nutritional safety concern (AgrEvo, 1997) in regard to glucosinolates.

Rape plants contain further inherent plant toxins and antinutritional factors such as phytic acid, which may limit the meal to be used in animal feed and/or human food and which also have to be considered for a safety assessment (Plant Genetic Systems, 1996). An evaluation of oilseed rape seed samples provided values from 4.68 to 6.01% phytic acid (defatted) for different modified lines compared to values between 4.82 and 6.16 for the control lines (Plant Genetic Systems, 1996) (see Table 3).

Comparisons between HCN92 and traditional canola counterparts showed that the typical phytate concentration of traditional canola meal (10% moisture basis) is between 3 and 6%. All canola evaluated in this study had less than 4% phytate content (HCN92: 3.240% oil-free basis, standards: 3.262–3.540%), and there was no statistical difference between cultivars tested (AgrEvo, 1992) (see Table 3).

Detailed data on glucosinolate and phytate levels in different modified rape plants are given in Tables 2 and 3.

Maize

The genetic modification of maize often aims at herbicide or insecticide resistance. Phytic acid occurs in considerable amounts in maize, and should therefore be analysed for an assessment of substantial equivalence of modified plants.

For a genetically modified insect protected maize line (Northrup King Co., 1996), the analysis shows that phosphorus, the most abundant inorganic component in maize, is largely present as the potassium-magnesium salt of phytic acid. But in respect of this constituent, no specific data are given in the dossier of this modified maize plant (Northrup King Co., 1996). For comparison of the genetically modified and the parent plant, only starch, protein, oil and fibre were analysed. Toxicity studies were performed on the expressed proteins but not on an eventually altered expression of key components.

The silage and grain of glufosinate tolerant corns (GTC) (AgrEvo, 1995) was found not to be different from current commercial varieties in essential nutrients or inherent plant toxins and antinutrients. All silage evaluated in the study had less than 0.15% phytate and there was no statistical difference between GTC and its non-transgenic counterparts. The mean phytic acid amount of GTC was about 0.07% dry weight, the mean of the non-transgenic counterpart about 0.055% dry weight (AgrEvo, 1995).

In preliminary documents of the genetically modified maize line GA21, tolerant to glyphosate herbicide (Monsanto, 1998), compositional components, such as protein, fat, ash, carbohydrates, moisture and fibre, amino acid composition and fatty acid profile, calcium and phoshorus were analysed, but no inherent plant toxins and antinutrients, such as phytate, were included.

In a genetically modified corn plant that controls European corn borer (DEKALB, 1996, 1997), the gene sequence that has been used for the modification shows a site which could encode for a protease inhibitor (chymotrypsin-inhibitor) acting as an antinutrient. As it is not clear whether this chymotrypsin inhibitor could be expressed in the plant after an integration in the genome, accurate investigations have been addressed on the behaviour of this gene site.

Molecular evidence demonstrates that important parts of the "chymotrypsin-inhibitory site" coding sequence has been deleted in the course of the insertion event, and analysis of plant tissues both support the conclusion that no transgenic chymotrypsin-inhibitor protein is produced in the modified maize lines. Lack of chymotrypsin-inhibitor protein in the modified maize has also been demonstrated by showing that the levels of endogenous protease inhibitor activity in the modified lines are the same as in non-transgenic plants. There is no evidence for any increase in chymotrypsin-inhibitory activity in any tissues.

Data also show that transgenic and non-transgenic kernels posses equivalent inhibitory activity (DEKALB, 1996, 1997).

Table 3. Content of glucosinolates (μmol/g meal) and phytate (%) in different modified rape plants or Canola

	Total glucosinolates		Phytate (%)	
Product	Modified	Control	Modified	Control
Glufosinate-tolerant canola HCN28 (AgrEvo USA Co., and AgrEvo Canada Inc., 1997)	12.4	9.8–19.6		
HCN92 (AgrEvo, 1992)	5.0–8.0	up to 17.1	3.240	3.262–3.540
New hybridization system (Plant Genetic Systems, 1996)	See Table 2		4.68–6.01	4.82–6.16

In this dossier (DEKALB, 1996, 1997), no data could be found on phytate levels. For the compositional analysis of the modified maize grain, only protein, oil, fibre, ash, moisture, amino acids and fatty acids were investigated.

Tomato

The improvement of genetically modified tomatoes now available is their shortened ripening time. Tomatoes, and other members of the genus *Solanaceae*, have the potential to accumulate deleterious secondary plant constituents known as glycoalkaloids. α-Tomatine is the principal inherent plant toxin/antinutrient in tomatoes. Although it has been isolated from all organs of the plant, it is routinely determined only by synthesis and degradation in the fruit. The level of α-tomatine decreases through fruit maturation and red ripe tomatoes loose almost all their tomatine when left on the plant for 2–3 days. Solanine and chaconine are the principal alkaloids of potato, but have been found in tomato in lower amounts. In tomatoes also considerable amounts of oxalate can occur (Souci *et al*., 1999).

If a genetic modification causes shorter ripening time the levels of tomatine, which decrease through maturation, could be influenced. So the assessment of this inherent plant toxin/antinutrient is very important but also the other factors such as solanine, chaconine, lectins and oxalate have to be controlled.

Genetically modified tomatoes intended for processing (especially for tomato paste) were analysed (Zeneca, 1996) for their levels of α-tomatine, solanine and chaconine in both the genetically modified fresh fruit and paste samples (Zeneca, 1996) (see Table 4). The results show that the glycoalkaloid levels in the modified tomato paste fall well within the range of glycoalkaloid levels of commercially available pastes, and the genetic modification has not altered the levels of the glycoalkaloids in the paste made from modified tomatoes. The analysis of tomato paste samples showed in line TGT7 58 mg α-tomatine/kg for the modified line, the unmodified plants had 74 mg/kg. All the other lines and also the fresh fruit samples showed less than 15 mg/kg tomatine. The amounts of α-chaconine and α-solanine were below the limit of detection of 5 mg/kg (see Table 4).

Besides the agglutination activity of tomato *seeds* caused by lectin, more recently the highest activity has been observed from the *juice* of ripe tomato fruits.

For the notification documents, analysis was carried out to find out whether any lectins were present in the modified paste. None of the modified paste samples showed lectin activity above the limit of detection, which is probably due to inactivation during processing (see Table 4) (Zeneca, 1996).

Saponins have not been tested for the purposes of this submission, because in tomato, saponins are mainly located in the seeds (Zeneca, 1996). No data on the oxalate were found. For an overview of contents of α-tomatine, α-chaconine and α-solanine and lectins, see Table 4.

Potato

Potatoes are genetically modified to achieve a changed starch composition such as an enhanced amylopektin fraction, or resistance to insects. Potatoes are known to contain the inherent plant toxins and antinutrients solanine and other glykoalkaloids, but furthermore, several protease-inhibitors or phenols (e.g. chlorogenic acid) are also present. Submitting a modified potato would need to show that the genetic modification had not, for instance, inadvertently increased alkaloid levels (Butler and Reichhardt, 1999).

Genetically modified starch potatoes with altered starch composition (Amylogene, 1996) were analysed for glycoalkaloid and chlorogenic acid content. The amount of glycoalkaloids can vary for different reasons, for example cultivar differences, yield, stage of tissue development and different types of stress (Amylogene, 1996). The genetic modification is not supposed to influence the content of these substances, and this was verified in the analyses executed: from the statistical analysis it is concluded that the amount of chlorogenic acid is not affected by the genetic modification (Amylogene, 1996) (see Table 5).

There were no significant differences in glycoalkaloid levels between different clones, but in a later reply-letter it was stated that the contents of glycoalkaloids are significantly smaller in the trans-

Table 4. Content of α-tomatine, α-chaconine, α-solanine (mg/kg) and lectin in modified tomato

	α-Tomatine		α-Chaconine and α-solanine		Lectin	
Sample (Zeneca, 1996)	Modified	Control	Modified	Control	Modified	Control
TGT7	58	74	< 5[1]	< 5[1]		
Other lines	< 15	< 15	< 5[1]	< 5[1]		
Fresh fruit	< 15	< 15	< 5[1]	< 5[1]		
Paste					na	na

[1]Below the limit of detection of 5 mg/kg. na = no lectin activity above the limit of detection.

Table 5. Content of chlorogenic acid, solanine, chaconine, total glycoalkaloids and trypsin-inhibitors in modified potatoes

Antinutrient	Starch potato (Amylogene, 1996)		Amylopektin potato (Avebe, 1996)		Literature (Füllgraff, 1989)
	Modified	Control	Modified	Control	Max recommended
Chlorogenic acid	66[1]	81[1]			
Solanine	94[2]	98[2]	50[3]	64[3]	20–40/100–200[5]
Chaconine	240[2]	221[2]	30[3]	74[3]	
Total glycoalkaloids	334[2]	319[2]	35[3]	45[3]	
Trypsin-inhibitors			1.5[4]	1.2[4]	

[1]μmol/100 g.[2]mg/kg dry weight.[3]μg/g product as is.[4]mg trypsin-inhibitors/g.[5]mg/kg. Edible potatoes should not contain more than 20–40 mg solanine/kg (other authors provide 100–200 mg/kg)

formed potato than in the recipient variety (Amylogene, 1996) (see Table 5).

In summary, it was stated that there are no increased contents of any of the inherent plant toxins and antinutritional substances examined (Amylogene, 1996).

Nutritional and toxicological consequences of a genetic modification of potato in respect to the amylopectin content were investigated (Avebe, 1996). The analysis on inherent plant toxins and antinutritional factors showed that the genetically modification did not change the total glycoalkaloid content in the potato, but the composition of the individual alkaloids could have been changed (Avebe, 1996). Analyses of feed for a subchronic oral toxicity trial with rats with modified and control potatoes did not reveal noticeable differences between the total alkaloids of the different diets (Avebe, 1996) (see Table 5).

Furthermore, protease-inhibitors, especially trypsin-inhibitors, were specified in the analyses of the feed for the subchronic trial (Avebe, 1996) (see Table 5). It is only mentioned that the *in vitro* trypsin-inhibitor activity in unheated potatoes is considerably lower compared to that in toasted soybeans, which are also used in livestock feed.

Other inherent plant toxins and antinutrients such as phenolic compounds and coumarins are not considered relevant in these documents and therefore they were not tested (Avebe, 1996). For detailed data on the contents of chlorogenic acid, solanine, chaconine, total glycoalkaloids and trypsin inhibitors in different modified potatoes, see Table 5.

Also, transgenic potato plants containing genes encoding for different classes of potentially insecticidal plant proteins, namely lectins, α-amylase inhibitors and chitinases, have been investigated (Gatehouse *et al.*, 1997). High levels of expression of the foreign proteins, which act as inherent plant toxins and antinutrients, were readily achieved throughout the leaf and stem tissue, and in the tubers. The expression of the lectin in transgenic potato plants caused significant detrimental effects to larvae (Gatehouse *et al.*, 1997).

Recently, data were published about genetically modified potato lines expressing the gene of snowdrop bulb lectin (GNA) (Ewen and Pusztai, 1999; Pusztai, 1998). In preliminary rat feeding trials the transgenic potatoes induced significant changes in the weights of some or most of the rats' vital organs, especially immune organs. Analysis shows that the contents of some of the constituents of major nutritional importance in these genetically modified potatoes are significantly different from those of their respective parent lines: protein and starch and/or glucose contents were different, similar findings were made for constituents such as lectin and trypsin- and chymotrypsin-inhibitors. The changes in major components in potato tubers after GNA-gene insertion and decreased foliar glycoalkaloid content in various lines of genetically modified potatoes may have occurred by mechanisms such as gene silencing, suppression and/or somaclonal variation as a result of gene insertion. Results have been discussed controversially, and an audit committee was of the opinion that the existing data do not support any suggestion that the consumption by rats of transgenic potatoes expressing GNA has an effect on growth, organ development or immune function (Bourne *et al.*, 1998). In any case, the results show that there is a lack of equivalence in composition between parental and modified potatoes which affects metabolic consequences of feeding (Pusztai, 1998).

Soybean

The most important modification of soybeans is tolerance against herbicides, but the development of new crops that have improved fatty acid patterns has also been successful.

Soybeans contain most diverse inherent plant toxins and antinutrients such as protease-inhibitors, lectins, isoflavones and phytate, which can have various deleterious effects for humans and animals when used as food or feed. Therefore, in the assessment of substantial equivalence, it is very important to consider these substances.

For the food and feed safety assessment of genetically modified glyphosate-tolerant soybeans, natural soybean constituents were measured in seeds (trypsin-inhibitor, lectins, iosflavones) and in toasted soybean meal (trypsin-inhibitor, lectins, iosflavones and phytate), and comparisons with the parental control indicated substantial equivalence

(Monsanto, 1994; Padgette *et al*., 1996). Analysis indicated that there were no significant differences in trypsin-inhibitor content between glyphosate-tolerant soybeans seeds and the control soybeans (see Table 6).

As processing soybean protein significantly inactivates trypsin-inhibitor, the level of trypsin-inhibitor in the toasted soybean meal from modified and control soybeans was measured. The toasting process resulted in a significant reduction in trypsin-inhibitor activity of the toasted meal relative to the seed. The trypsin-inhibitor levels in the toasted meal lots analysed were all comparable to or lower than the values reported in the literature. The processing caused a reduction of trypsin-inhibitor from 45 to 3 trypsin-inhibitor units (TIU)/mg dry weight for the glyphosate-tolerant soybeans and from 43 to 3 TIU/mg sample dry weight for the control (see Table 6).

There were also no significant differences in lectin activity; the levels were even found to be very low in the soybean seeds—lower than previously reported for other soybean lines. The glyphosate-tolerant soybeans had a similar quantity of lectin activity to the control soybeans (Monsanto, 1994; Padgette *et al*., 1996) (see Table 6).

The levels of lectins in the toasted meal samples were below the detectable limits. For the modified soybeans, a reduction from 6 to less than 0.5 haemagglutinating units (HU)/mg extracted protein resulted from the processing. The contents in control lines were reduced from approximately 7 to less than 0.5 HU/mg extracted protein (see Table 6). Although the seed lectin values measured were lower than reported in the literature, these results do show that toasting does significantly reduce lectin activity, both in the glyphosate-tolerant soybeans and control lines (Monsanto, 1994; Padgette *et al*., 1996).

No statistical differences in the content of isoflavones (genistein, daidzein, coumestrol and biochanin A) in the seeds were detected between the modified and the control soybeans (Monsanto, 1994; Padgette *et al*., 1996).

The amounts of isoflavones in glyphosate-tolerant soybeans toasted meal batches were equivalent to the control soybean toasted meal batch, as expected, since no differences were found in the whole seeds. The phytate concentration in the glyphosate-tolerant soybeans toasted meal samples were similar to those in the control samples and was claimed to be substantially equivalent (Monsanto, 1994; Padgette *et al*., 1996) (see Table 6).

The compositional analysis of a new transgenic soybean variety which produces a soybean oil with a dramatically modified fatty acid spectrum (high oleic acid transgenic soybean) (Du Pont, 1996) included a comparison of the soybean seed from high oleic lines with the parent variety (control) in order to determine that there were no unexpected changes in composition. As antinutritional factors trypsin-inhibitors and phytic acid were investigated (Du Pont, 1996) (see Table 6). No differences in these two components were observed between control and high oleic soybeans (Du Pont, 1996). But in this dossier no data on lectins and isoflavones were provided.

For detailed data on trypsin-inhibitor, lectin and phytate-content in modified soybeans see Table 6.

DISCUSSION

Genetic modification of food- or feed-related plants is developing rapidly, but until now no long-term experience on ecological or nutritional effects is available. There is an apparent lack of studies dealing with the long-term risks of plant biotechnology (Butler and Reichhardt, 1999). Ecological science tries to evaluate what really constitutes ecological risks and what methods can be applied to identify and quantify those risks (for review, see Hails, 2000). In the field of nutritional risk assessment, especially the relevance of pleiotropic effects is unclear, but could play a role in the question of possible changes in the expression of constituents such as inherent plant toxins and antinutrients. The impossibility of a prediction of the integration

Table 6. Trypsin-inhibitor, lectin and phytate content in different modified soybeans

	Trypsin-inhibitor		Lectin (HU/mg prot.)		Phytate (g/100 g dry weight)	
Product	Modified	Control	Modified	Control	Modified	Control
Glyphosate tolerant soybean	45[1]/23.7[3]	43[1]/22.6[3]	5.6–6.6[4]	6.3[5]		
Toasted	3[1]	3[1]	< 0.5(nd)	< 0.5(nd)	1.81–1.93	1.76–1.91
High oleat soybean	40–47[2]	51–62[2]			1.25–1.55	1.3–1.4
Lit. (Belitz and Grosch, 1992; Lang, 1979; Souci *et al*., 1999)	31–42[3] raw					1.0–1.5
Lit.	6.4–93.2[1]/16.7–27.2[3]					
Toasted meal	3.8–17.9[1]					1.3–4.1

[1](TIU/mg dry weight). [2](TIU/g). [3](mg TI/g). [4]5.6–6.6 haemagglutinating units (=HU)/mg protein extract = 2.6–3.2 HU/mg total protein = 1.0–1.2 HU/mg sample. [5]6,3 HU/mg protein extract = 3.0 HU/mg total protein = 1.2 HU/mg sample. nd = below the detectable limit of 0.5 HU.

region of the genetic modification is one of the main problems for the estimation of the probability and of the dimension of pleiotropic effects (Koschatzky and Massfeller, 1994). If the location of the DNA insertion in the genome of the host organism is not fully known, and it cannot be assumed that in view of the location of the insert there will be no harmful effects, tests should be carried out that include an evaluation of possible changes in known macro- and micronutrients and relevant non-nutrient constituents such as inherent plant toxins and antinutrient factors. If these analytical tests indicate no major differences in the levels of well-known key constituents, it may be considered that the chance of other metabolic alterations leading to the production of significant amounts of, for example, other inherent plant toxins and antinutrients will be unlikely (Belitz and Grosch, 1992).

The concept of substantial equivalence is used internationally for the nutritional and toxicological risk assessment of GMOs used for novel foods (or feeds). Recently this concept was criticized and biological, toxicological and immunological tests rather than merely chemical ones were demanded (Millstone *et al*., 1999), but strong scientific support as responses to this point of view defended the use of this principle. Even though an assessment of substantial equivalence is necessary for notification, the analysis of selected documents of genetically modified plants indicates that often relevant data in regard to inherent plant toxins and antinutrients are missing. Although some reviewed documents may still be preliminary or some crops may not be intended for immediate use as food or feedstuff, a general lack of information was evident. Some documents provided no data at all about inherent plant toxins and antinutrients, and argue that the analysis of some constituents is not relevant. In fact, there are no coherent regulations to which companies can adhere for the selection of inherent plant toxins and antinutrients that have to be analysed. The documents show no consistency, but without providing comparable data, an assessment of substantial equivalence cannot be conclusive.

In several cases significant differences between the glucosinolate content of modified rape lines and the non-transgenic counterparts were observed. In results of local experiments the glucosinolate contents of the transgenic lines are clearly above the non-transformed plants and furthermore far above the recommended standard for canola seed. Many of them are also above the allowed quality standard for oilseed meal. Some explanations given in the documents for the interpretation of results of different experiments seem critical: the results may be reflected by local environmental conditions, and documents suggest that normal agricultural breeding practices might ensure recommended glucosinolate levels. It is furthermore claimed that it is generally accepted that commercial plant-derived food exhibit considerable variability in their composition (Plant Genetic Systems, 1996). But this conjecture may not be scientifically justified: how much is the accepted range of the variability, what are the regulations for variations above the statistical limits, what variations may be dangerous? These questions do not seem to be completely clarified yet. In one experiment in the UK (Plant Genetic Systems, 1996), where the contents of glucosinolates are alarming, these values are attributed to drought stress, and the presented data suggest that environmental factors have a major impact on the seed quality characteristics compared to the genotype. Further experiments are necessary for use of the rape plants as accepted food. Even though it is admitted in the documents that in some cases the glucosinolate levels per gram of seed and per gram of meal are significantly higher in the transformed rape seeds than in the control (Plant Genetic Systems, 1996), this is not explained sufficiently.

In this study, inherent plant toxins and antinutritive compounds have been analysed for an assessment of potential effects of interventions in genetically modified plants. These constituents have been selected since inherent plant toxins and antinutrients are of great importance in a nutritional analysis; these compounds are a recent topic of controversial scientific discussion and not many data are available on their effective concentrations. Until now, no internationally agreed ranges for their acceptable concentrations and variations are given. It remains unclear for many inherent plant toxins and antinutritional compounds, which variations could also cause nutritional effects in a population, and statistical methods to evaluate significance remain to be specified.

The amounts and natural variation of antinutritive substances in one plant species can differ considerably, since they are influenced by many factors: state of ripening; year of production; storage; varietal differences; and growing conditions (climate, soil quality), and also stress or pathogen infection (Ene-Obong, 1995; Teuscher, 1994). Literature data sometimes show very wide variations in typical concentrations of inherent plant toxins and antinutrients (Füllgraff, 1989). When the substantial equivalence of GMOs with their parental organism is analysed, this natural variation in content of inherent plant toxins and antinutrients has to be taken into consideration.

Although ranges for most of the compositional variables are available in the literature, these data may not be directly comparable due to differences in analytical methods or sample preparation. In addition, much of the literature data are relatively old and may not completely encompass the compositional variables of modern crop varieties (Koschatzky and Massfeller, 1994).

A further problem is the comparison between different studies, especially the parameters of analysis such as units and bases of standardization of substances, which show no conformity and are difficult to interpret.

Special care has to be taken in case genes encoding inherent plant toxins and antinutrients are the target of the genetic modification. The expression of such proteins is certainly lucrative for agriculture because it often confers new paths of resistances, but long-term effects on human health and the environment are not easily assessed. In general, genetic modifications enclosing known toxins or antinutrients may be problematic and should be postponed until all uncertainties of the risk assessment can be assessed adequately.

In conclusion, the present review proves the usefulness of the concept of the substantial equivalence in risk assessment. A major present problem is the lack of specification as to which key components have to be analysed in a certain genetically modified plant to establish substantial equivalence. Until now there are no coherent regulations that specify which inherent plant toxins and antinutrients have to be declared and tested in which plant. A minimum list of macro- and micronutrients as well as secondary plant constituents, inherent toxicants, and allergens that should be analysed in order to assess substantial equivalence has to be agreed for specific crops. This list should include a total proximate analysis (protein, fat, ash and moisture) together with those key nutrients or key antinutrients, key toxicants and key allergens known to be associated with the crop. This selection will also need to take into account the way in which the crop is to be processed and consumed as well as the dietary needs of the consuming population (Nordic Council of Ministers, 1998). Papers discussing variations of food composition are to be anticipated (biotechnologies and food: Assuring the safety of foods produced by genetic modifications. IFBC, *Regulatory Toxicology and Pharmacology*, Vol. 12, No. 3, December 1990, part 2 of 2 parts) or consensus documents such as "on key nutrients and key toxicants in canola oil and canola meal" (OECD document ENV/JM/FOOD(99)4), or could serve as a starting point for such a work.

Furthermore, a difficult problem for an assessment of substantial equivalence is the fact that it is not always technically possible to use authentic isogene control lines. Moreover, the process to obtain all the required permission takes a long time, and in the end the compared data from transgenic lines often come from very early transformants and are not really consistent with the final products. A conclusive analysis of the substantial equivalence of a new genetically modified plant is complex and time-consuming work especially because of the wide natural variations and the fast progressing breeding programs and techniques, such as gene stacking. Special care has to be taken in investigating and controlling possible effects of environmental conditions on constituents of genetically modified crops. Although such effects, possibly caused by unusual environmental parameters, can similarly be seen with conventional crops several problems to agronomic relevant properties have been observed in genetically modified crops, due, for instance, to unusual temperatures and possible changes in plant physiology caused by the addition of genes (Fox, 1996; Kaiser, 1996). Such observations, together with a specific public awareness, indicates that conclusive information requirements for notifications and a scientifically reviewed, public available risk assessment, as well as post-marketing controls are important to establish gene technology in the production of food and feedstuff.

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