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Substantial equivalence of antinutrients in GMOs used for 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 potential pleiotropic effect of genetic modifications on constituents of modified crops data from US and EC documents were investigated in regard to antinutrients. Analysed were documents of rape (glucosinolates, phytate), maize (phytate), tomato (tomatine, solanine, chaconine, lectins, oxalate), potato (solanine, chaconine, protease-inhibitors, phenols) and soybean (proteaseinhibitors, lectins, isoflavones, phytate). In several documents used for notifications no declarations even on essential antinutrients could be found, e.g. data on phytate in modified maize were provided only in one of four documents. Significant variations in contents of antinutrients in parental and modified plants especially due to environmental influences were frequently observed: Drought stress, e.g. was made responsible for significantly increased glucosinolate levels of up to 72.6 $\mu\text{mol/g}$ meal in modified and parental rape plants in field trials compared to recommended standard concentrations of less than 30 $\mu\text{mol/g}$. Taking into account these wide natural variations generally the concentrations of antinutrients in modified products were in the range of the concentrations in parental organisms. The presented results on antinutrients indicate that the concept of the substantial equivalence is useful for the risk assessment of GMOs used for novel foods but possible environmental influences on constituents of modified crops need more attention. Consistent guide-lines, 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 antinutrients on nutritional safety, also coherent databases of standard parental lines and clear criteria for mandatory declarations are necessary.

Introduction

At present, 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 GMOs according to directive 90/220/EEC. In the US the Food and Drug Administration controls the food and feed safety of genetically modified organism (GMOs) by the guidance “Foods Derived From New Plant Varieties”.

In the EC all GMOs which 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 case of demonstrated substantial equivalence of key-toxic or allergenic compounds, key-nutrients and possible antinutrients and a risk-assessment of the genetic modification, the modified product can be placed on the European market with a notification only [14]. Therefore, the assessment of the substantial equivalent is not only important for the risk assessment but decides also on regulatory decisions. Special attention in the analysis of substantial equivalence has to be focused on antinutritive constituents, as genetic modification could effect the expression of gene products not addressed by the genetic modification (unintentional pleiotropic effects) and thereby alter the content of constituents such as antinutrients [23].

Still not entirely harmonised is the definition of plant inherent toxicants and antinutrients. Usually antinutrients are understood as substances that inhibit or block important pathways in the metabolism, especially the digestion. Antinutrients reduce the maximum utilisation of nutrients

(esp. 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 [35]. Only substances with primary effects on the availability of nutrients are considered in this article but not compounds with only toxicological qualities. However, many antinutrients may also be toxic beyond a certain dose e.g. oxalate or cyanogenic acid.

Most of the deleterious effects of antinutrients are caused by raw plant material. Most of the antinutritive substances become ineffective by simple measures like heating, soaking, germination or autoclaving. Recently also data about positive effects of antinutritive substances have been published, e.g. anticancerogene and antibactericidal qualities were found [35]. A list of the most frequent and important classes of antinutrients, their occurrence and their nutritional effects is given in Table 1.

The expression of constituents of crops such as antinutrients and thereby their concentrations in a genetically modified plant can eventually be influenced by pleiotropic effects [20]. Such effects can occur when the integration of the genetic material into the genome leads to non predictable phenotypical 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 [23]. Regulatory elements in the plant DNA can influence the expression of the inserted genes and random insertion events may 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 [24].

In 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 [23]. Therefore, aspects of pleiotropic effects have to be taken seriously in the assessment of the substantial equivalence. As antinutrients are important constituents in the assessment of the substantial equivalence of key nutrients the expression of antinutrients have been compared in documents of genetically modified crops. This analysis therefore focuses on possible effects of genetic interventions on antinutrient constituents and should not be misunderstood as a safety assessment..

Material and Methods

For a comparative analysis of the content of antinutrients in genetically modified and parental plants, we evaluated data of antinutrient 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 antinutrients in plants [7, 24, 33] the following antinutrients 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, proteaseinhibitors and phenols, and soybeans for proteaseinhibitors, 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 are public widely available on internet servers or registers of national competent authorities e.g.:

<http://www.aphis.usda.gov/biotech/>

<http://www.maff.gov.uk/food/>

<http://biosafety.ihe.be/>

Results

It was found that in most genetically modified organisms the levels of antinutrients were within the range of the non-transformed parental organisms. In several documents no or only incomplete data on antinutrients could be found. Until now, there are no specific regulations that specify which antinutrients have to be declared and tested in which plant and consequently there is also no consistency according to that companies can proceed for their analysis of antinutrients. Some documents provided no data at all about antinutrients, or conclude that some antinutrients are not relevant e.g. 1 saponins in tomato [36] or phenolic compounds and coumarins in potato [6]. Only in one of four documents of modified maize plants the antinutrient phytate was analysed. In the investigated dossier of modified tomato, many antinutrients were tested like glykoalkaloids and lectins, but no data were provided about oxalate [36]. In regard to modified soybeans, the analysis of lectins and isoflavones are controversial, in one dossier their levels are indeed determined [30], in another not [12]. Also the determination of antinutrients in potato are not coherent, data about chlorogenic acid are provided in one document [5], in another not [6]; the same problem was found with trypsin-inhibitors.

In one dossier of modified rape plants [31] 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 [5], 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 antinutrients, oilseed rape contains glucosinolates, and also phytic acid in the oilfree meal [13]. Erucic acid is not regarded as an antinutrient, but it would have to be analysed in an assessment of toxicology of the oil. Several documents of notification dossiers of genetically modified rape plants [1, 3, 31] have been analysed for data on antinutrients:

In one document of genetically modified rape plants [31] 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 that it is generally accepted that commercial food derived from plants exhibit considerable variability in their 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 (less than 20 μmol alkenyls/g oilfree meal). In the documents it is affirmed that the collected data of the genetic

modified lines fit within the range established for oilseed rape [31]. But it is also stated that the biochemical analysis data of transformed and non-transformed seeds show that e.g. in releases in Belgium the glucosinolates per gram of seed and per gram of meal are significantly higher than the control [31].

Quality standards for oilseed rape meal allow not more than 30 μmol of total glucosinolates (total of gluconapin, glucobrassicinapin, 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 8-73 μmol glucosinolates/g oilfree meal, and for seeds between 11-42 μmol glucosinolates/g oilfree seed (see Tab.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 $\mu\text{mol/g}$ meal (see Tab. 2). In one seed quality analysis experiment (FBN9501) values were between 66.56 and 72.62 μmol glucosinolates/g *meal* for the transformed lines, which is high above the quality standard of 30 $\mu\text{mol/g}$, and also high levels were found in the seeds of 37.89-41.59 μmol glucosinolates/g *seed* (see Tab.2). The similar increase of the glucosinolate level of all entries is said to be caused by drought stress.

But 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 [31].

Canola is a trademark term that is presently defined as seed, oil and meal from *B. napus* and *B. rapa* plants that contain no more than 30 μmoles of aliphatic glucosinolates/g of oil-free, moisture-free meal [3].

Analysis of two notifications of glufosinate tolerant canola crops which have been released in 1997 (HCN28) and 1995 (HCN92) [1,3], show without exception that glucosinolate levels are less than 20 $\mu\text{mol/g}$. HCN28 meal consistently had glucosinolate levels of 12.4 $\mu\text{mol/g}$ or less. HCN92 had levels of 5.0-8.0 $\mu\text{mol/g}$ (see Tab. 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 [1, 3]; thus documents conclude that HCN28 does not present a nutritional safety concern [3] in regard to glucosinolates.

Rape plants contain further antinutritional factors like phytic acid which may limit the meal to be used in animal feed and/or human food and which have to be considered too for a safety assessment [31]. 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 to 6.16 for the control lines [31] (see Tab. 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 % oilfree basis, standards: 3.262 to 3.540 %) and there was no statistical difference between cultivars tested [1] (see Tab. 3).

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

MAIZE

The genetic modification of maize often aims at herbicide- or insecticide-resistance. The antinutrient 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 [29], 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 to this antinutrient, no specific data are given in the dossier of this modified maize plant [29]. 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) [2] was found not to be different from current commercial varieties in essential nutrients or antinutrients. All silage evaluated in the study had less than 0.15 % phytate and there was no statistical difference between GTC and its nontransgenic counterparts. The mean phytic acid amount of GTC was about 0.07 % dry weight, the mean of the nontransgenic counterpart about 0.055 % dry weight [2].

In preliminary documents of the genetically modified maize line GA21, tolerant to glyphosate herbicide [29], compositional components, like protein, fat, ash, carbohydrates, moisture and fibre, amino acid composition and fatty acid profile, calcium and phosphorus were analysed, but no antinutrients, like phytate, were included.

In a genetically modified corn plant (DBT418) that controls European corn borer [10, 11], 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 if 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 demonstrate that important parts of the “chymotrypsin-inhibitory site” coding sequence has been deleted in the course of the DBT418 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 maize plants containing the DBT418 insertion event are the same as in non-transgenic plants. There is no evidence for any increase in chymotrypsin-inhibitory activity in any DBT418 tissues. Data also show that transgenic and non-transgenic kernels posses equivalent inhibitory activity [10, 11]. In this dossier [10, 11] no data could be found on phytate levels. For the compositional analysis of the DBT418 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 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 lose 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 [33].

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

Genetically modified tomatoes intended for processing (especially for tomato paste) were analysed [36] for their levels of α -tomatine, solanine and chaconine in both the genetically modified fresh fruit and paste samples [36] (see Tab. 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/kg α -tomatine 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 Tab. 4).

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

For the notification documents, it was analysed if 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 Tab. 4) [36].

Saponins have not been tested for the purposes of this submission, because in tomato, saponins are mainly located in the seeds [36]. No data on the antinutrient oxalate were found.

For an overview of contents of α -tomatine, α -chaconine and α -solanine and lectins see Tab. 4.

Potato

Potatoes are genetically modified to achieve a changed starch composition like an enhanced amylopectin fraction, or resistance to insects. Potatoes are known to contain the antinutrient solanine and other glycoalkaloids, but furthermore, several proteaseinhibitors or phenols (like chlorogenic acid) are also present. Submitting a modified potato would though need to show that the genetic modification had not inadvertently increased alkaloid levels, for instance [23].

Genetically modified starch potatoes with altered starch composition [5] were analysed for glycoalkaloid and chlorogenic acid content. The amount of glycoalkaloids can vary due to different reasons, e.g. cultivar differences, yield, stage of tissue development and different types of stress [5]. The genetic modification is not supposed to influence the content of these substances, and that 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 [5] (see Tab. 5).

There were no significant differences in glycoalkaloid-levels between different clones but in a later reply-letter it was said that the contents of glycoalkaloids are significantly smaller in the transformed potato than in the recipient variety [5] (see Tab. 5).

In summary, it was stated that there are no increased contents of any of the antinutritional substances examined [5].

Nutritional and toxicological consequences of a genetic modification of potato in respect to the amylopectin content were investigated [6]. The analysis on antinutritional factors showed that the genetic modification did not change the total glycoalkaloid content in the potato, but the composition of the individual alkaloids could have been changed [6]. Analyses of feed for a sub-

chronic oral toxicity trial with rats with modified and control potatoes did not reveal noticeable differences between the total alkaloids of the different diets [6] (see Tab. 5).

As further antinutrient, protease-inhibitors, especially trypsin-inhibitors were specified in the analyses of the feed for the sub-chronic trial [6] (see Tab. 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 used in livestock feed too.

Other antinutrients like phenolic compounds and coumarins are not considered relevant in these documents and therefore they were not tested [6]. For detailed data on contents of chlorogenic acid, solanine, chaconine, total glycoalkaloids and trypsin-inhibitors in different modified potatoes, see Tab. 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 [18]. High levels of expression of the foreign proteins, which act as 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 [18].

Recently data were published about genetically modified potato lines expressing the gene of snowdrop bulb lectin (GNA) [15, 32]. 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 antinutrient contents like 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, 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 [8]. In any case, the results show that there is a lack of equivalence in composition between parental and modified potatoes which effects metabolic consequences of feeding [32].

SOYBEAN

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

Soybeans contain most diverse antinutrients like proteaseinhibitors, lectins, isoflavones and phytate which can have various deleterious effects for humans and for 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 with antinutritional activity 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 [26,30]. Analysis indicated that there were no significant differences in trypsin-inhibitor content between glyphosate-tolerant soybeans seeds and the control soybeans (see Tab. 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 TIU/mg DW for the glyphosate-tolerant soybeans and from 43 to 3 TIU/mg sample DW for the control (see Tab. 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 [26,30] (see Tab. 6).

The levels of lectins in the toasted meal samples were below the detectable limits. For the modified soybeans, a reduction from 6 to <0.5 H.U/mg extracted protein resulted by the processing. The contents in control lines were reduced from ca. 7 to <0.5 H.U/mg extracted protein (see Tab. 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, in both the glyphosate-tolerant soybeans and control lines [26,30].

No statistical differences in content of isoflavones (genistein, daidzein, coumestrol and biochanin A) in the seeds were detected between the modified and the control soybeans [26,30].

The amounts of isoflavones in glyphosate-tolerant soybeans toasted meal batches were equivalent to the control soybean toasted meal batch, as expected, since there were no differences 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 [26,30] (see Tab. 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) [12] 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 [12] (see Tab. 6). No differences in these two antinutritional components were observed between control and high oleic soybeans [12]. 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 Tab. 6.

Discussion

Genetic modification of food or feed related plants is developing rapidly but until now no long-time experiences on ecological or nutritional effects are available. There is an apparent lack of studies dealing with the long-term risks of plant biotechnology [9]. Ecological science tries to evaluate what really constitutes ecological risks and what methods can be applied to identify and quantify those risks [for review: 19]. 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 antinutrients. The impossibility of a prediction of the integration region of the genetic modification is one of the main problems for the estimation of the probability and of the dimension of pleiotropic effects [23]. 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 like 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 e.g. other antinutrients will be unlikely [7].

The concept of substantial equivalent is used internationally for the nutritional and toxicological risk assessment of genetically modified organisms used for novel foods (or feeds). Recently this concept was criticised and biological-, toxicological and immunological tests rather than merely chemical ones were demanded [25], 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 antinutrients are missing. Although some reviewed documents may still be preliminary or some crops may not be intended for immediate use as food or feed stuff a general lack of information was evident. Some documents provided no data at all about antinutrients, and argue that the analysis of some antinutrients are not relevant. In fact there are no coherent regulations companies can adhere to for the selection of antinutrients that have to be analyzed. The documents show no consistency. But without providing comparable data, an assessment of substantial equivalence can not 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 highly above the recommended standard for canola seed. Many of them are also above the allowed quality standard for oilseed meal [33]. 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 [33]. 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? Those questions do not seem to be completely clarified now. In one experiment in the UK [31], where the contents of glucosinolates are alarmingly, 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 a use of the rape plants as accepted food. Even though in the documents it is admitted 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 [31], this is not explained sufficiently.

In this study antinutritive compounds have been analysed for an assessment of potential effects of interventions in genetically modified plants. These constituents have been selected, as 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. Unclear for many antinutritional compounds remain also the variations which could cause nutritional effects in a population.

The amounts and natural variation of antinutritive substances in one plant species can differ considerably, as they are influenced by many factors: State of ripening, year of production, storage, varietal differences and growing conditions (climate, soil quality) but also stress or pathogen-infection [17, 33]. Literature data show sometimes very wide variations in typical concentrations of antinutrients [17]. When the substantial equivalence of GMOs with their parental organism is analysed, this natural variations in content of antinutrients have 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 is relatively old and may not completely encompass the compositional variables of modern crop varieties [23].

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

Special care has to be taken in the case that genes encoding antinutrients are target of the genetic modification. The expression of such proteins is certainly lucrative for agriculture because it often confers new ways of resistances , but long term effects on humans health and the environment are not easily to be 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 specifications what are the key-components which have to be analysed in a certain genetically modified plant to establish substantial equivalence. Until now there are no coherent regulations that specify which 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 [28]. Consensus documents such as “on key- nutrients and key- toxicants in canola oil and canola meal” (OECD document ENV/JM/FOOD(99)4) 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 get all permission needed takes a long time and at 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 a 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 e.g. 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 e.g. due to unusual temperatures and possible changes in plant physiology caused by the addition of genes [16, 22]. Such observations together with the fact of an specific public awareness indicate 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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Tab. 1: Classes of the most frequent antinutrients

| ANTINUTRIENT | OCCURRENCE | EFFECT |
|--|---|--|
| Cyanogenetic Glycosides | Maniok, cassava, yams, sweet potato, fruit (stones), millet, phaseolus lunatus, limabean, | Blocking of cell-breathing, gas Influence on carbohydrates and Ca- doses iodine-deficiency |
| Glucosinolates (Goitrogene): Sinapsin, Sinigrin, Progoitrin, Arachidosid | Cruciferae, esp. in seeds: rape, mustardseeds, radish, cabbage, kale, peanut, soybean, onion, cassava | Strumatic effects (forming of goitre) Thyroxin synthesis ↓, metabolis. absorption ↓, protein digestion ↓ |
| Glykoalkaloids (Solanine and Tomatine) | potato, tomato (Solanaceae), unripe fruit | Inhibition of Cholinesterase; gas Hämolysis, inflammation of kidney |
| Gossypol | Cottonseeds | Binds metals, iron absorption ↓, inhi |
| Lectins (Phyto-Haemagglutinines) | Fabaceae, cereals, soybean, beans | Inflammation and damage of the resorption of nutrients and N-Reto protein-synthesis), ↓ enzyme activ resorption |
| Oxalate | Spinach, celeriac, beetroot, rhubarb, Amaranth, silver beet, tomato | Ca-oxalate-crystals, insoluble salts resorbable) → Ca-metabolism impair |
| Phenols (flavonoids, Isoflavone, chlorogen acid) | Vegetables, fruit, vine, cereals, soybean, potato, tea, coffee, plant oils | Destruction or inhibition of thia availability of trace elements hypocholesterolemic activities |
| Phytate | all plant-seeds, cereals, fabaceae | Complexes: Bioavailability of Ca, utilisation of protein and starch ↓ (C and amyolytic enzymes) |
| Protease-inhibitors | Fabaceae-seeds, peanut, cereals, rice, maize, batate, potato, apple | Inhibition of trypsin and chymotry and Pankreaselastase → ↓ digestion |
| Saponin | Fabaceae, spinach, asparagus, sugar-beet, soybean, tea, peanut | Complexes with proteins and lip haemolytic, Gastroenteritis, most sap |
| Tannins | Widespread: all fruits, tea, coffee, vicia faba | Inhibition of pancreatic enzymes, Thiamine utilisation ↓, Availability of |

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Tab. 2: exemplary contents of glucosinolates [μ moles/g seed or meal] in modified rape

| New Hybridization System [26] μ mol 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

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Tab. 2: exemplary contents of glucosinolates [μ moles/g seed or meal] in modified rape [26].

Tab. 3: Content of glucosinolates [μ moles/g meal] and phytate [%] in different modified rape plants or Canola

| Product | Total glucosinolates | | Phytate [%] | |
|--------------------------------------|----------------------|------------|-------------|-------------|
| | modified | control | modified | control |
| Glufosinate tolerant Canola | | | | |
| HCN28 [3] | 12.4 | 9.8-19.6 | | |
| HCN92 [1] | 5.0-8.0 | up to 17.1 | 3.240 | 3.262-3.540 |
| New Hybridization System [26] | See Tab. 2 | | 4.68-6.01 | 4.82-6.16 |

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Tab. 3: Content of glucosinolates [μ moles/g meal] and phytate [%] in different modified rape plants or Canola

Tab. 4: Content α -tomatine, α -Chaconine, α -solanine [mg/kg] and lectin in modified tomato

| Sample [31] | α -tomatine | | α -Chaconine and α -solanine | | Lectin | |
|-------------|--------------------|---------|--|-----------------|----------|---------|
| | 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

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Tab. 4: Content α -tomatine, α -Chaconine, α -solanine [mg/kg] and lectin in modified tomato .

Tab. 5: content of chlorogenic acid, solanine, chaconine, total glycoalkaloids and trypsin-inhibitors in modified potatoes .

| Antinutrient | Starch potato [5] | | Amylopektin potato [6] | | Literature [15] |
|----------------------|-------------------|------------------|------------------------|------------------|------------------------------|
| | 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 $\mu\text{mol}/100\text{ g}$

² $\text{mg}/\text{kg DW}$

³ $\mu\text{g}/\text{g}$ product as is

⁴ $\text{mg trypsin-inhibitors}/\text{g}$

⁵ mg/kg . Consumptionalbe potatoes should not contain more than 20-40 $\text{mg Solanine}/\text{kg}$
(others authors provide 100-200 mg/kg)

Waltraud Novak: Substantial equivalence of antinutrients in genetically modified novel food.

Tab. 5: content of chlorogenic acid, solanine, chaconine, total glycoalkaloids and trypsin-inhibitors in modified potatoes

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

| Product | Trypsin-inhibitor | | Lectin [H.U/mg prot.] | | Phytate [g/100g DW] | |
|-----------------------------------|---|--|-----------------------------------|-------------------------------|---------------------|-----------|
| | Modified | Control | Modified | Control | Modified | control |
| Glyphosate [25] Toasted | 45 ¹ / 23.7 ³ 3 ¹ | 43 ¹ / 22.6 ³ 3 ¹ | 5.6-6.6 ⁴ <0.5 (nd) | 6.3 ⁵ <0.5 (nd) | 1.81-1.93 | 1.76-1.91 |
| High oleat [12] | 40-47 ² | 51-62 ² | | | 1.25-1.55 | 1.3-1.4 |
| Lit. [7, 20, 29] | | 31-42 ³ raw | | | | 1.0-1.5 |
| Lit. [28] Toasted meal | | 26.4-93.2 ¹ / 16.7-27.2 ³ 3.8-17.9 ¹ | | | | 1.3-4.1 |

¹ [TIU/mg DW]

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 H.U

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Tab. 6: Trypsin-inhibitor, lectin and phytate content in different modified soybeans].