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EVALUATION OF STRATEGIES FOR FOOD SAFETY ASSESSMENT OF GENETICALLY MODIFIED AGRICULTURAL PRODUCTS

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ABSTRACT

Evaluation of strategies for food safety assessment of genetically modified agricultural products.

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The use of gene technology has enabled the plant breeder to introduce foreign genes from evolutionary far related species into existing selection lines. These lines can thereby be improved with respect to a variety of (quality) traits.
The rapid progress of these scientific developments has urged regulatory authorities to consider the necessity of additional regulation for novel food products derived from these genetically modified plants. A number of advisory reports has already been published on the issue. So far, the FDA (USA) is the only regulatory institution that has published a statement of policy.
In this report risk evaluation strategies as proposed by national and international bodies, either issued or under development, are reviewed and suggestions are made for further improvement of risk evaluation procedures for genetically modified complex products.

Keywords: food safety regulation, genetic modification, plant breeding

PREFACE

New technologies entail new questions. The greater the power of the new technology the more numerous the questions will be. Recombinant-DNA techniques are not an exception to this rule. In theory, the number of possible applications of these new techniques is almost unlimited as is the number of problems to be foreseen when the techniques are applied. In practice, the number of applications is rather limited (for the time being) and it is seems to be possible (in the near future) to perform a reliable risk assessment of the new products obtained in this way. Such a risk assessment does not have to yield to risk assessment procedures, if available, for new products obtained by means of classical breeding.
In this report an inventarisation is made of possible applications of genetic modification in agriculture and (proposed) strategies for adequate food safety assessment of these products. Also a number of bottle-necks for optimal risk assessment procedures is discussed.

This report is written within the framework of the RIKILT-DLO project 'Risk analysis on novel foods for the consumer' as part of the 'Ecological, social and ethical aspects of biotechnology' program financed by the Department of Science and Technology of the Dutch Ministry of Agriculture, Nature Conservation and Fisheries.

SUMMARY

Recent developments in gene technology have enabled plant breeders to establish genetic combinations that could not be obtained by means of classical breeding procedures. Thereby it has proved feasible to improve a number of (qualitative) characteristics in several plant species. Examples of such improved characteristics by means of genetic modification are disease resistance, insect resistance, frost resistance, prolonged shelf-life and storage quality, nutritional value and sensoric characteristics. Other traits that can be modified in this way are a.o. herbicide resistance and male sterility.

Because of these developments in plant breeding national and international regulatory bodies have considered the question as to how adequate existing regulation is with respect to the food safety aspects of novel foods derived from genetically modified organisms. This has resulted in a number of advisory reports proposing different strategies for risk assessment. Important international reports in this respect are the IFBC (International Food Biotechnology Council) and FAO/WHO reports and the EC and OECD (draft) reports. Proposals for national regulation have been published in Great Britain and the Netherlands. In addition a Skandinavian advisory organ has published guidelines. So far only the United States has formulated official guidelines. In this report the different strategies to assess the food safety of genetically modified agricultural products are evaluated and suggestions are made for further improvement of risk evaluation procedures for genetically modified complex products.

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1 APPLICATIONS OF GENETIC MODIFICATION

1.1 Introduction

In a remarkably short space of time, recombinant-DNA techniques have found a wide range of applications within the plant breeding sector. The most frequently used application is the detection of specific DNA sequences that code for or correlate with a desirable production trait. In the first case it is the gene itself that causes the trait, while in the second a bordering sequence or a functionally related gene is involved. Within the framework of several research networks, gene maps of important food crops, such as tomato and potato, are currently being made in various places. Because the presence of desirable traits can now be determined at any stage of development, insight into genetic structure at the DNA level can be helpful to plant breeders in accelerating selection procedures. Whereas in the past one needed fully grown plants in order to be able to test for traits such as disease resistance or yield, it is now possible, at least if the specific trait is DNA-characterised, to perform a simple DNA test during the germination phase.

However, the most remarkable application of recombinant-DNA techniques in the plant breeding industry is the genetic modification of food plants. Genetic modification is possible using genes that are already naturally present in the plant (cisgenesis) and which are only artificially amplified. However, most applications of genetic modification relate to the introduction of foreign genes of vegetable, bacterial, animal or even human origin (transgenesis). The DNA code is universal and therefore, in principle, exchangeable between all living organisms. Dialects, however, do occur with the code and for the sake of increased expression levels it is sometimes desirable to 'translate' the DNA from the donor organism to the code of the acceptor organism before introducing the DNA segment into the genome of the acceptor.

Several different transformation systems for establishing genetic modification are currently available [Lindsey et al., 1989]. The most frequently used, and so far the most efficient, is the system that makes use of the bacterium Agrobacterium tumefaciens. This bacterium contains a plasmid with a transposable element which, when it infects vegetable material, can be transferred to the genetic material of the plant. By substituting part of this transposon by a gene coding for a desirable trait it has proved possible to transfer a desirable trait into the DNA of plant cells. Apart from the Agrobacterium tumefaciens system, other systems have been developed, such as electroporation, micro-injection and a system that uses micro-projectiles to genetically modify plant cells. However, these alternative systems, at least in dicotyledons have not proved to be as efficient as the Agrobacterium tumefaciens system.

Other important techniques that are applied to establish genetic modification (but where there is no direct modification) are somaclonal variation and protoplast fusion. Somaclonal variation is a phenomenon related to cell culture procedures. Genetic variation can occur as a result of spontaneous mutations at already existing loci, but the possibility that new genes may arise in this way cannot be excluded [Cocking, 1990]. However, in a number of species it has proved to be rather difficult to grow new plants from the cultured cells. Using protoplast fusion, transgenic plants that combine characteristics of strains or species that cannot be crossed in the classical way can be obtained. Protoplast fusion can be established under the influence of high voltage or by means of polyethylene glycol (PEG). The fusion results in a heterokaryon that can subsequently grow into a plant. For a long time now, protoplast fusion has been the only available method to genetically modify monocotyledonous species, such as rye or rice [Van der Maas et al., 1990; Hensgens et al., 1990]. The extremely low efficiency of heterokarya to grow into plants has been a major problem in plant breeding for many years [Cocking, 1990; Lazzeri et al., 1990]. It is not only possible to introduce foreign genetic material into the nucleus but, indirectly, it also seems possible to produce transgenic mitochondria by means of tRNA of nuclear sequences [Small et al., 1992]. This technique, however, has not yet been fully developed and it is still not clear whether the relatively large DNA fragments, necessary to accomplish an effect, can be introduced in this way.

The list of transformable food plants is growing steadily. Although a considerable number of genetic modification experiments in plants are still being performed for fundamental research purposes, the number of experiments directed to improve certain production characteristics of economically important food plant varieties is increasing. So far these improvements have been limited to those characteristics coded for by just one characterised and localised gene. Therefore, the number of agronomically important traits that can be improved by genetic modification is still rather small, because the majority of interesting traits are based on a combined action of several genes at the same time.

1.2 Disease resistance

Virus resistance is the trait that is most often introduced by genetic modification. Virus resistance has been obtained in this way for example in tobacco, tomato, potato, melon, sugar beet, soy beans, alfalfa, artichokes and rice. Resistance to various viruses, such as the alfalfa mosaic virus, cucumber mosaic virus, potato viruses X (PVX) and Y (PVY) and potato leaf roll virus can be achieved [Nelson et al., 1990; Fraley, 1992]. Furthermore, experiments to introduce virus resistance in cassava are in progress [Persley, 1990]. To establish resistance, several genes coding for viral proteins have been

introduced into the genome of these species. This strategy has proved successful in several cases, although the mechanism that leads to resistance is not yet fully understood.
Other methods of obtaining virus resistance have been developed, incorporating other viral sequences not coding for coat proteins. For example, resistance to the tobacco mosaic virus has been achieved by incorporating part of the replicase gene of the virus [Fraley, 1992]. However, recent experiments have shown that resistance obtained in this way can be nullified when the introduced DNA fragment recombines with a defective virus that is lacking in the transgenic sequences. The consequences for the biosafety of plant varieties which have viral sequences added to their genome are not clear [Gal et al., 1992; Allison et al., 1990; Creamer et al., 1990]. Other strategies possibly leading to virus resistance are, for example, the introduction of antisense viral transcripts, expression of antiviral antibodies or expression of interferon [Gadani et al., 1990]. So far these methods have not resulted in effective resistance. A certain degree of resistance to the fungi Alternaria longipes and Rhizoctonia spp. has been achieved by incorporating the bacterial chitinase gene into tomato, potato, lettuce and sugar beet [Fraley, 1992; Jones, 1992]. In addition, experiments are being done to obtain resistance to bacterial diseases by introducing several antibacterial agents derived from insects (e.g. sarcotoxins, apidaecins, cecropins), vertebrates (magainins) or plants (thionins) [Florack et al., 1990]. Transgenic tobacco plants containing the gene for acetyltransferase have been shown to be resistant to wild fire, caused by Pseudomonas syringae.

1.3 Insect resistance

Several methods have been developed to make commercial plant varieties resistant to a series of voracious insects. The one most often applied involves the introduction of a gene that codes for a crystalline protein of the bacterium Bacillus thuringiensis. Researchers from Plant Genetic Systems (Belgium) were the first to introduce insect resistance in tobacco plants by incorporating the Bt-gene. This protein exerts its insecticidal action specifically with respect to the larvae of several harmful insects. Many B. thuringiensis strains have been characterised, all of which show specific toxicity to certain insect families. Most strains are active against the larvae of specific butterfly species. Others are also active against Diptera or Coleoptera species. For many strains, the specific activity still needs to be determined [Goré et al., 1986]. The gene that codes for the active toxin has been incorporated in tomato, potato, walnut, rice, corn and cotton, etc. [Harlander, 1990; Perlak et al., 1990] and has been shown to be rather effective against the voracity of several larvae. Furthermore, it has been found that effectivity increases with improved expression as a result of 'translation' of the bacterial DNA into a code that is more easily recognised in plant cells [Perlak et al., 1990].

Another way of introducing insect resistance in plants is to use proteinase inhibitors. These compounds occur naturally in Leguminosae and cereals and are thus already present in substantial amounts in the human diet. The inhibitor that has hitherto been used most often in genetic modification experiments is the cowpea trypsin inhibitor [Hilder et al., 1990]. In transgenic plants expression of these proteinase inhibitors needs to be relatively high in order to exert the desired effect. The fact that inhibitors cannot exert their insect resistant effect in the absence of other toxic factors can, therefore, not be excluded. Proteinase inhibitors have been introduced for example in tobacco and potato. In these species insect resistance can also be achieved by introducing lectin genes (haemagglutinins) [Edwards et al., 1991]. Lectins are abundant in nature, especially in the seeds of Leguminosae. They are capable of binding carbohydrates specifically, but the precise mechanism of insect resistance still needs to be clarified.

1.4 Frost resistance

Experiments are currently being performed to determine the effects of freezing and thawing on the composition and taste of transgenic tomatoes into which a gene has been introduced that codes for an anti-freezing protein. The protein is derived from the winter flounder which, with the aid of this protein, protects itself against freezing in the Arctic seas. If the experiments are successful the next step will be to make strawberries frost-resistant in a similar way [Jones, 1992]. Some of the first field experiments were concerned with genetically modified bacteria in which the 'ice' gene was lacking. This gene codes for a protein which catalyses the formation of ice crystals under specific environmental conditions. It was shown that when plants are sprayed with ice-bacteria a certain degree of frost resistance can be achieved.

1.5 Herbicide resistance

Resistance to several herbicides has been accomplished. This development can in principle result in the more widespread application of more effective or biologically degradable herbicides. Another way in which herbicide resistance genes can be applied is as marker genes in transformation experiments. This application is particularly important for research purposes.

Herbicide resistance occurs naturally in several plant species. It has been shown that this resistance is often caused by a single dominant mutation. Herbicide resistance can be achieved by modifying the expression level and sensitivity of the target enzyme of the herbicide or by introducing a gene that causes detoxification of the herbicide into the plant genome [Oxtoby et al., 1990]. The first method is mainly applied with respect to herbicides interacting with plant photosynthesis, for example triazins,

or amino acid synthesis, for example glyphosate (Roundup), phosphinotricin (Basta), sulphonylureas and imidazolinones. Triazin resistance (atrazin) has been achieved in tobacco, and experiments are currently being performed on Brassica and Solanum strains. Experiments are also in progress to achieve resistance to the photosynthesis inhibitor Bromoxynil by introducing the bacterial bxn gene into tobacco and tomato plants. The bxn gene that codes for nitrilase is capable of detoxifying Bromoxynil. In a similar way, attempts are being made to introduce phosphinotricin resistance into tobacco, potato and tomato by means of the bacterial bar gene that codes for an acetyltransferase. Many herbicides are rapidly degraded by soil bacteria. These bacteria are therefore a rich source of herbicide resistance genes. Glyphosate tolerance, using target enzyme modification, has been achieved in tobacco, tomato and Petunia. Phosphinotricin, sulphonylurea and imidazolinone resistance has been accomplished in a similar way in tobacco, soy beans and maize. Finally, experiments are currently being done using tobacco plants to introduce the bacterial tfdA gene in order to obtain resistance to the herbicide 2,4-D, which is a growth inhibitor. The target enzyme of 2,4-D has not yet been identified [Oxtoby et al., 1990; Llewellyn et al., 1990; Fraley, 1992].

1.6 Shelf-life and storage quality

Improved storage quality and prolonged shelf-life have been achieved by the inactivation of specific genes. This inactivation is brought about by introducing antisense sequences. In tomato, introducing the antisense sequence of the gene coding for the polygalacturonase enzyme has resulted in the almost complete disappearance of this specific enzyme in ripening tomatoes [Lindsey, 1991; Grierson et al., 1990]. In an alternative strategy to prolong shelf-life and improve the storage quality of tomatoes, the antisense sequence of 1-aminocyclopropane-1-carboxylate (ACC) or of ACC-oxidase (both enzymes being involved in ethylene synthesis) has been introduced [Lindsey, 1991; Fraley, 1992; Jones, 1992]. Although it is generally assumed that effective translation is inhibited by hybridisation of the sense and antisense RNA fragments, the exact mechanism behind the effectivity of the antisense sequences is not yet fully understood [Holden, 1990]. Modification of flower colour in Petunia is based on inactivation of the chalconsynthase enzyme involved in flavonoid synthesis [Lindsey, 1991]. In this case, no antisense genes are involved but inactivation is caused by an unknown interaction between several chalconsynthase genes present. Another technique being developed and which should lead to the inactivation of specific genes, makes use of genetically modified ribozymes. Ribozymes are RNA particles with extremely specific endoribonuclease activity [Holden, 1990]. Specific ribozymes can be developed for specific mRNA sequences, thereby inhibiting the translation of these sequences into protein.

1.7 Male sterility

Male sterility has been achieved in rapeseed, tobacco, lettuce, chicory, cotton, tomato and maize by introducing a specific promotor joined to a fungus-derived RNAse. The promotor is tissue (tapetum cell layer in the anthers) and development stage specific [Rochaix, 1992]. Introducing the gene coding for this RNAse inhibits pollen formation which results in sterile male plants, which can subsequently be used in classical breeding strategies to obtain hybrid plants with the desired characteristics. Fertility of these plants can, if necessary, be restored by introducing a specific RNAse inhibitor in plants of the same variety. Crossing these two transgenic strains will result in some fertile plants.

1.8 Nutritional composition

Modifications in the metabolism of a plant can lead to improved nutritional composition. One example of this is the modification of the metabolism of rapeseed resulting in modified fatty acid chains with an increased nutritional value [Amer. Medic. Ass., 1991]. Nutritional characteristics can also be improved by introducing nonsense proteins with a favourable amino acid composition, by expression of specific genes [Lindsey et al., 1989] or by eliminating harmful metabolites in specific plant varieties. Research to improve the amino acid production of potatoes and the amino acid composition of cassava by introducing synthetic genes is currently being carried out [Persley, 1990]. Furthermore, it has been shown that it is possible to increase starch production in potatoes by twenty to forty per cent by expressing the bacterial ADP-glucose-pyrophosphorylase gene under the control of a tissue-specific patatin promotor [Fraley, 1992; Rochaix, 1992]. A higher starch level will reduce the costs of processing and lead to decreased fat absorption during frying. Increased sucrose and reduced starch levels have been obtained by introducing the sucrose phosphate synthase gene. Increasing the expression of certain genes can be achieved by replacing the traditional promotor by one with a higher activity or by one that causes prolonged expression of the gene in the development of the plant or in organs other than the traditional ones. However, only very few tissue or developmental stage specific promotors have yet been identified. Besides modifications in metabolism for nutritional purposes other modifications in metabolism have been accomplished that serve industrial applications (agrification).

1.9 Sensory characteristics

Experiments to improve the sensory characteristics of plant products are performed with genes coding for, amongst others, thaumatin and monellin. These proteins are the sweetest compounds known at present. They are derived from the plants Thaumatococcus daniellii and Dioscoreophyllum cumminsii, respectively. Thaumatin genes have been expressed in potato [Jones, 1992]. Monellin has been introduced into tomato and lettuce [Penarrubia et al., 1992]. It is expected that these natural non-sugar sweeteners will find wider application in the near future.

2 GUIDELINES

2.1 Introduction

We have seen from the previous chapter that there are many ways of modifying specific characteristics of organisms by means of genetic modification. Modification can imply the introduction of a gene (product), but also modified expression or inactivation. These forms of genetic recombination are now applied quite extensively in micro-organisms and plants and to a somewhat lesser extent in animals. Examples of applications in the food industry are the production of food ingredients, such as sweeteners, dietary acids and enzymes by micro-organisms, increased production of food plants because of increased disease resistance and possibly, in the future, the production of lean meat.

Crop improvement with the aid of genetic modification offers a number of advantages when compared with traditional breeding. The most important advantages related to the technique are:

- the precision of the modification;
- the ability to cross traditional borders in order to establish favourable genetic combinations.

There are, however, some restrictions when applying the technique:

- the available transformation systems cannot yet be applied to every organism;
- for higher organisms it is not yet possible to site the gene into the genome with sufficient accuracy.

An additional problem both in classical as well as in 'molecular' breeding is the limited knowledge of physiology and composition in the different organisms before and after breeding procedures. These factors are important for the risk assessment of genetically modified food products.

At present, genetically modified products are largely in the research or development phase. However, it is to be expected that some of these products will soon be offered on the market. Efforts are therefore being made to evaluate the risk to the consumer of such products. In the past few years several proposals have been published for (inter)national regulation with respect to the food safety of genetically modified agricultural products. The proposed guidelines are all intended to safeguard the safety of the consumer, while hampering free trade traffic as little as possible.

The following organisations have already published proposals for regulation:

* International Food Biotechnology Council (1990)
* Scandinavia: Nordic Working Group on Food Toxicology and Risk Assessment (NNT, 1991)
* United Kingdom: Advisory Committee on Novel Foods and Processes (ACNFP, 1991)
* FAO/WHO (1991)

* Netherlands: Health Council (1992)

In addition the following organisations have published draft guidelines:

* Netherlands: Food Council
* EEC: DG III Internal Market and Industrial Affairs
* United States: the Food and Drug Administration (FDA) is the only government institution that has so far published official guidelines in a 'Statement of Policy: Foods derived from new plant varieties' in 1992. These guidelines refer exclusively to new plant strains.

An important common factor in the different proposals, with the exception of the EEC draft proposal, is the rejection of specific legislation for food products derived from genetically modified organisms. It is generally felt that these new food products can be evaluated within the framework of existing legislation which only requires specific clarification on a few points.

In the following chapters, the different guidelines formulated so far and the (draft) proposals for regulation will be discussed briefly, together with an analysis of agreements and differences. This review of proposed guidelines is not exhaustive. A selection, representing the different views, has been made. For example, in 1990 the 'Food Sanitation Investigation Council' of the Japanese Committee for Biotechnology published draft guidelines for products obtained by recombinant-DNA techniques [Food Sanitation Invest. Council, 1990]. These guidelines, however, only relate to products of genetically modified organisms in those cases where the modified organism itself does not enter the food chain. Furthermore, in 1989 the 'Technical Committee on Novel Foods' of ILSI Europe published a draft discussion paper on the subject in 1989 [ILSI, 1989]. These proposals can be found in the list of references.

2.2 International Food Biotechnology Council (IFBC)

The IFBC was established in 1988 and consists of some 30 (bio)technological companies in the food producing industry. One of the reasons for setting up the Council was the need for the food producing industry to have guidelines in order to be able to meet future requirements with respect to the safety evaluation of novel food products [Lindemann, 1990]. In 1990, the Council published the report 'Biotechnologies and food: assuring the safety of foods produced by genetic modification' [IFBC, 1990]. This document deals extensively with the risk evaluation of genetically modified food products. The aim of the Council was to set up scientific criteria for the evaluation of the safety of products or ingredients derived from genetically modified plants or micro-organisms. Applications of biotechnology in animal production are not considered.

Figure. 1 Genetic modification of food products and guidelines in relation to the food safety of these products.

1973	First successful transformation experiment in micro-organisms (E. coli)
1982	First successful transformation experiment in mammals (mouse)
1988	First successful transformation experiment in plants (tobacco) Admission of the first GMO derived product onto the market (Switzerland: Kluyveromyces lactis derived chymosin).
1990	Admission of the first GMO onto the market (Great Britain: Bakers' yeast) Calgene Inc. submits a report on the food safety aspects of the kanamycin resistance gene to the FDA as a request for advisory opinion. Publication of the IFBC (International Food Biotechnology Council) report on the food safety of GMO derived products.
1991	Publication of reports on the food safety aspects of GMO derived products by Scandinavian and British advisory committees and by FAO/WHO. Calgene Inc. submits a report on the food safety aspects of a transgenic tomato to the FDA as a request for advisory opinion.
1992	Publication of the report on the food safety aspects of GMO derived products by the Dutch Health Council. Publication of the FDA guidelines for the food safety aspects of new plant varieties.

The IFBC report includes a historical review of food production and food safety evaluation through the ages and a chapter on classical and novel genetic modification techniques used in plant breeding. An elaborate survey of present knowledge on composition with respect to macro and micro-nutrients, natural toxins and antinutritional factors (ANFs) in crop plants is also included. An important starting-point for the IFBC is that sufficient knowledge of the composition of most food products is already available, so that the altered composition of genetically modified products can be investigated. For risk evaluation the following bottle-necks are discussed:

- the use of antibiotic resistance genes as marker genes in transformation experiments. In agreement with the Calgene report on the same subject [Calgene, 1990], it is stated that the use of antibiotic resistance genes does not cause any risks unless heavy selection pressure is exercised on, for example, micro-organisms in the GI tract that have incorporated such an antibiotic resistance gene by horizontal transfer from the plant genome. This is more deeply discussed in Chapter 3.
- pleiotropic effects, including position effects with respect to expression levels and insertion in coding and non-coding sequences. It is stated that the usual laboratory and field experiments will bring possible pleiotropic effects to light. The fact that transposons and chromosomal recombination are natural phenomena show that pleiotropic effects are not uniquely related to novel genetic modification techniques.

- changes in the genetic code as a result of tissue cultures. With respect to this phenomenon it is also stated that the usual procedures offer enough opportunity to demonstrate possible deleterious genotypic changes.

The IFBC has set up three decision trees for different product categories in order to determine the kind of information to be supplied for the product safety evaluation. These decision trees successively relate to:

- Food ingredients derived from micro-organisms.

Risk analysis of these food products and ingredients should mainly focus on the genetic background of the organisms and constructs used, and on the existing knowledge concerning their toxicity. In those cases where it is known that both organism and construct do not code for pathogenic and/or toxic elements, then the genetically modified organism is considered to be safe. Other factors that are of importance and should be determined, are the genetic stability of the producing organism and the influence of process variables and regulating elements. The decision tree also indicates that no antibiotic resistance genes should be present in the final organism.

- Single chemicals and simple mixtures.

In contrast to whole food products, single substances and simple chemical mixtures can be relatively well characterised. Usually, standard toxicity testing will sufficiently safeguard the safety of food additives, micro-nutrients, residues and contaminants. One example of a substance produced by a genetically modified micro-organism is the enzyme chymosin. Both new and permitted food ingredients, produced by means of recombinant-DNA techniques are considered safe if they contain no toxic or unknown components and if future exposure does not exceed existing limits. For this reason the genetic background of both construct and production organism need to be well characterised.

- Whole foods and other complex mixtures.

Complex food products and macro-ingredients should also be basically regulated within the framework of existing procedures and regulations as applied to comparable classical food products and ingredients. Therefore, a genetically modified tomato that has incorporated an anti-sense polygalacturonase gene sequence in order to improve storage quality should, in principle, be regulated in the same way as a traditionally bred tomato. Evaluation should focus on the genetic make-up and composition of the product, when known and different from the traditional counterpart, and on the estimated exposure. Knowledge concerning the genetic make-up implies thorough characterisation of the product and complete analysis and characterisation of the construct, which should not code for toxic elements. Animal toxicity testing should only be performed in very specific cases, also in view of the anticipated difficulties with the practicability and interpretation of such studies. The combination of having to compose a balanced diet and the application of the safety

factors usually used in standard toxicity testing, is especially likely to cause major problems. Possible changes in composition can be determined by analytical research on nutrients, natural toxins and factors that may interfere with the processing of the product. When evaluating the product one should bear in mind the considerable variation in components that can exist within a species and at a specific developmental stage (ripening, storage). For example, the levels of tomatin can vary considerably depending on the stage of ripening of the tomato. An extra paragraph of the IFBC report is devoted to standard toxicity testing of complex products and the problems to be anticipated when performing these studies.

In conclusion it can be stated that the IFBC approach to the safety evaluation of novel foods is a case-by-case approach focusing on the product. The technique used to obtain the product is not considered to be of major importance when evaluating the product as such.

Finally, the report provides a survey on present American legislation for (edible parts) of plants and plant products, processed plants, other biological ingredients, processing substances and chemical additives. The IFBC states that the evaluation of novel foods obtained by means of genetic modification should primarily take place within the framework of existing procedures. New plant varieties should, in principle, not be registered. Notification of genetically modified varieties should be done on a voluntary basis.

2.3 Scandinavia - Nordic Working Group on Food Toxicology and Risk Assessment.

In 1988, the Scandinavian Advisory Committee on Food Problems (in which Denmark, Finland, Iceland, Norway and Sweden participate) set up the Nordic Working Group on Food Toxicology and Risk Assessment (NNT). In 1991, this working group published the report 'Food and new biotechnology - novelty, safety and control aspects of foods made by new biotechnology' [Nordic Working Group, 1991].
The report provides a survey of new biotechnological techniques and assesses the impact of such techniques on the food producing industry [Klopper, 1991]. The influence of new techniques such as genetic modification and cell fusion on plants, micro-organisms and animals is analysed. Restrictions for applying genetic modification are observed in all three categories. These restrictions mainly relate to:

- limited knowledge of gene regulation
- lack of stable and efficient transformation systems
- lack of suitable cloned genes.

Because of the importance of plants in our daily consumption pattern, genetic modification in plant breeding will, in the future, affect human nutrition. The working group states, however, that the borderline between traditional and advanced biotechnology is not absolute and will therefore be difficult to regulate as such. It is anticipated that Solanaceae (potato, tobacco and tomato) and other important dicotyledonous crop plants such as rapeseed and sugar beet will be the first genetically modified species to appear on the market, followed by Leguminosae (e.g. soy beans) and the first cereals (rice and corn). With respect to genetic modification in animals, the working group expects transgenic fish to be the first to enter the market. The reason for this assumption is that genetic modification in fish would encounter fewer ethical scruples.

With respect to the safety of these new products when compared with their traditional counterparts, it is stated that in the latter (although assumed to be safe) high levels of natural toxins often cause acute or chronic toxic symptoms. The report provides an elaborate though not exhaustive survey on natural toxins. Within a species, large variations with respect to nutrient and toxin composition are often observed. According to the report, new biotechnological techniques may have a beneficial effect on, amongst other things, the concentration of natural toxins.

Risk evaluation of food products derived from genetically modified organisms should be based on the following three principles:

1. Insertion of a genetic construct has an effect comparable to mutagenesis. The insertion as such does not cause specific problems.
2. The biochemical characteristics as well as the expression pattern of the newly introduced gene product(s) are decisive for risk evaluation.
3. The new expression products may indirectly affect the metabolism of the host organism. Examples of such interference are downstream effects after insertion, and competition for amino acids.

When evaluating risk, attention should also be given to genetic stability, genetic distance between the donor and host organism and the selection procedure. It must be emphasised that it is essential to have analytical references for both traditionally produced food products as well as for those developed via genetic modification.

The working group proposes to relate the risk evaluation of the new product to the relative novelty of the product. This can be determined by taking the present food supply as a reference. The working group suggests a case-by-case approach as the most appropriate. For the evaluation a four step procedure is set up, comparable to the decision trees proposed by other organisations. Each step can lead to acceptance, further experiments or rejection of the novel food.

The first step is to make an inventory of the knowledge of genetic background, composition and estimated exposure. Information with respect to composition should include data on levels of protein, peptides, fats and fatty acids, single and complex carbohydrates as well as on natural toxins and antinutritional factors and other components such as enzymes, minerals and vitamins. This information, which is rather comprehensive, serves to compare the new product with its traditional counterpart. If it is shown from this initial phase that the product cannot be considered as new it can be marketed directly. If, however, it is found to be a new product a risk evaluation will be performed on the introduced gene product and possible secondary changes in composition. The gene product will be evaluated according to the existing regulations for additives or contaminants.

If the final product is a complex one the next step will consist of non-human feeding trials and metabolism studies of the product, both in vitro and in vivo. A 90 day feeding trial in rats will in all cases form part of these studies. A safety factor of less than 100 in these toxicological studies will be acceptable whenever nutritional elements are studied. If antinutritional factors or toxins are involved traditional safety factors for additives will have to be applied. The final step will consist of experiments using human volunteers. These studies will focus on human allergy and intolerance. Finally, the working group recommends post-marketing research.
These draft procedures are illustrated by a few examples. In a transgenic tomato that has incorporated a gene coding for the coat protein of tobacco mosaic virus, the natural level of these proteins in non-modified tomatoes will need to be analysed in order to determine the novelty of the product. A transgenic carrot on the other hand that has incorporated a gene coding for a trypsin inhibitor into the genome will in all cases be considered as new, as this inhibitor does not naturally occur in carrot. In addition, the carrot is likely to be processed differently, compared with the donor organism of the inhibitor.

The report recommends the possibility of identifying products produced by genetic modification. The working group advises that prior to approval of new products an identification method will be recorded so that the product can be checked after marketing. For products that do not enter the food chain a suggested identification method is the incorporation of easily detectable markers in genetically modified plants or animals.

2.4 United Kingdom - Advisory Committee on Novel Foods and Processes

In 1988, the Advisory Committee on Novel Foods and Processes (ACNFP) was established in the United Kingdom. The predecessor to this advisory group was the Advisory Committee on Irradiated

and Novel Foods, established in 1980. The ACNFP published their advisory report 'Guidelines on the assessment of novel foods and processes' to the Ministers of Health and Agriculture of the United Kingdom and the Head of Departments for Health, Social Services and Agriculture of Northern Ireland in 1991. In this report, the Committee proposes guidelines for the producers of novel foods. For different product categories it is determined what information will have to be provided before approval of the product for the market can be considered.

The ACNFP uses a broad definition of novel products and processes: all food products and ingredients that have so far not been available for human consumption in the United Kingdom in significant amounts and all processes that are new or significantly altered for the production of food products. Products such as rapeseed oil and processes such as freezing and irradiation are therefore also subjected to the guidelines [Klopper, 1991]. If a product is obtained by making use of such a process the producer will have to notify the ACNFP. The ACNFP will subsequently perform a risk evaluation using the data provided by the producer.

The proposed guidelines focus on determining possible changes in composition, especially with respect to nutrients and natural toxins. The guidelines are intended for products obtained by means of classical breeding strategies as well as by genetic modification. So far, producers in the United Kingdom can still decide whether the safety of novel foods is sufficiently guaranteed and whether or not new products are submitted to the Committee.
The ACNFP has set up a decision tree to determine what information should be provided. This decision tree differs from those set up by the IFBC in a number of ways. First of all the ACNFP tree includes both products and processes. The products are not classified in advance in different categories, but pass through the same decision tree. Finally, the tree does not result in guidelines for the admission of the product onto the market, but determines what information should be provided by the producer. The information to be supplied for products derived from genetically modified organisms largely corresponds with the information as required in other proposals: genetic background of the organism, data with respect to composition, especially nutrients and natural toxins, processing method, estimated exposure, effects of the genetic modification on the metabolism of the host organism and possible transfer of the incorporated construct to other organisms. In addition, information on the stability of the genetically modified organism is explicitly required as well as a description of the tissues and organs where the incorporated genetic material is expressed. For example, an important factor when evaluating a genetically modified potato can be whether or not the incorporated gene is expressed in the potato tuber. In those cases where the estimated exposure shows that the new product will be consumed in large quantities human allergenicity studies should

be considered. *The advisory committee stresses that the decision tree is not intended to be a mere checklist. The committee advocates a flexible approach whereby it can be determined which information is relevant for the safety evaluation of each new product.*

The ACNFP therefore also proposes a case-by-case approach with clear guidelines in a single decision tree. The committee stresses the importance of transparency to the public and thus advises the risk evaluation procedures to be deposited in the British Library for public inspection. The report provides eight examples of the information required for the evaluation of novel foods according to the ACNFP decision tree. The examples vary from a new application of a well-known product produced by an approved process, to the introduction of genetically modified bakers' yeast.

In an appendix the report states that with respect to the labelling of products derived from genetically modified organisms the following categories should be distinguished:

- nature identical food products of genetically modified organisms
- foods from intra-species genetically modified organisms
- novel food products of genetically modified organisms
- foods from trans-species genetically modified organisms

Labelling should in principle be required for the last two categories. It should also be stressed that certain flexibility should be possible.

2.5 Food and Agriculture Organisation/World Health Organisation

In November 1990, a joint FAO/WHO consultation on the 'Assessment of biotechnology in food production and processing as related to food safety' took place in Geneva. In 1991, WHO published the conclusions of this meeting in a report [WHO/FAO, 1991].

One of the aims of the meeting was to make a first move towards international consensus with respect to guidelines to safeguard the safety of products obtained by genetic modification. This could serve as a basis for national regulation. The meeting in Geneva can be seen as the first in a series of activities to achieve this effect.
The report includes a survey of applications of genetic modification in 1) bacteria and fungi, 2) plants and 3) animals, followed by a consideration of the safety aspects.

For the first category, micro-organisms, the report mentions a number of factors that could influence the safety of the final product: the genetic background of the original, non-modified organism, the

characteristics of this organism and the methods used to introduce the desired traits. Functional marker genes will have to be removed in the final production organism. Intermediary organisms must also be well characterised. Food-grade vectors are now being developed for this purpose: vectors that can be used in food production without any health hazard. In this respect it is important that the genetic construct does not code for any toxic elements and that it is incorporated in a stable way.

The chance of (harmful) pleiotropic effects should not be increased when compared with traditional breeding. *A risk analysis concerning the effects of consumption on human health should be based on an analysis of pathogenicity, toxicity and possible changes in nutrient composition in comparison with the traditional counterpart.* The report also mentions the possibility of an increase in the number of allergic reactions by the introduction of new or modified proteins. Knowledge of the induction of allergic reactions is limited and no reliable methods are yet available to determine allergenicity.
Other factors that may affect safety are:

- the fermentation process including substrates and other substances present, and the growing conditions;
- the isolation procedure (and subsequent steps in the purification process);
- the final normalisation of the product.

It is important that in all cases production should occur under GMP conditions. It is stressed that in a group of products with a similar genetic background there is always some variation. This is even the case where single substances are concerned. This should be considered when evaluating the safety of novel foods.
The committee of experts advocates guidelines for novel foods which correspond with existing FAO/WHO guidelines for food additives, based on a case-by-case approach. The committee prefers general principles based on scientific research to specific regulation for different product categories. For micro-organisms this implies that if the genetically modified organism shows considerable resemblance to the traditional counterpart, relatively few additional experiments will be necessary.

In order to ask the right questions with respect to the safety of the second category of products, derived from genetically modified plants, it is important to be familiar with the applied technology and the effects of the genetic modification. Potential risks should be related to the risks as they occur in traditionally bred plants.

The report mentions some aspects that need to be addressed when evaluating the safety of genetically modified plants. The genetic construct and possible expression products must be safe. It is assumed that the genes incorporated in a stable way will reach a fixed and predictable expression pattern. Information about this expression pattern should be as detailed as possible.

In the report pleiotropic effects mainly refer to the metabolic effects of the introduced expression product. It is anticipated that the biochemistry of the expression product will generally be sufficiently characterised to predict these metabolic effects. Moreover, these aspects do not differ substantially from those in classical breeding. Similarly, insertional mutagenesis cannot be considered as a new phenomenon in this respect. The committee of experts states that a changed composition with respect to natural toxins and/or nutrients is in itself not sufficient reason to block the marketing of the product.

Compared with other reports on the subject the committee deals more specifically with possible toxicological and nutritional changes. Five possible toxicological changes are mentioned:

* the presence or increased content of natural toxicants;
* the presence of new expressed toxic materials resulting from genetic modifications (e.g. biopesticides);
* development of allergenicity;
* accumulation of toxicants or microbial contaminants derived from the environment;
* changes in the availability of toxins as a result of processing.

Changes in the nutritional value of the product can be the result of:

* modification of major nutrients, micronutrients or antinutrients in the food;
* changes in the bioavailability of these substances;
* changes in the nutritional components as a result of product processing.

It is thought that performing toxicological tests on complete, complex food products will cause major problems. Risk evaluation based on data of molecular, biological and chemical characteristics of the product is therefore preferable. Analysis of the product may imply research on protein and amino acid composition as well as the composition of the sugar and lipid fraction, inorganic components and vitamins, naturally occurring toxins and antinutritional factors and storage quality. If the food plant is a major constituent of the national diet, biological availability studies may be necessary. If the information thus obtained is insufficient for a proper food safety evaluation, animal studies or, eventually, human studies will need to be considered. The latter would imply food trials with volunteers or limited introduction onto the market. The technology used, classical or recently developed, will determine the amount and nature of the desired information.

For the third category of novel foods, those obtained from genetically modified animals, risk evaluation is presumed to be rather simple. Evaluation should mainly be based on risk assessment of the

expression product, the genetic construct and possible pleiotropic effects. Risk analysis of the expression product can be performed according to procedures used for an exogenously administered equivalent. The construct should be free of viral sequences, thereby reducing the risk of gene transfer to the gut flora. According to the working group, potential pleiotropic effects will probably come to light if they affect the health of the animals. Unacceptably high levels of toxins in healthy animals have not been recorded in the history of animal breeding and the chance of this occurring does not seem to increase substantially by applying genetic modification. In lower vertebrates and invertebrates, however, this chance is possibly more significant and should therefore be considered as a factor in the risk analysis of products derived from these animals.

In products from genetically modified animals it is important to determine possible changes in nutritional value. The composition of the novel food should therefore be analysed. The possible occurrence of food intolerance could also be an important factor. This could be caused by the introduction of foreign genes but also by the expression of chimeric genes as a result of the association of transgenic and endogenic sequences. In general it will not be necessary to perform toxicity tests on the complete product.

The report advocates a scientifically based case-by-case approach. With such a flexible approach it is possible to include the latest scientific developments in the evaluation. The report also signals the necessity to set up well-informed databases of constituent compounds of food products (nutrients and natural toxins) and of gene maps of organisms used in food production. Finally, the importance of an efficient supply of information to the consumer is emphasised, with respect to the application of biotechnology in food production as well as to the safety of such products.

2.6 Netherlands - Health Council and Food Council

In 1988, the Minister of Agriculture, Nature Conservation and Fisheries and the Parliamentary Secretary of State of the Ministry of Welfare, Health and Cultural Affairs presented a request for advice to the Food Council with respect to 1) aspects of new biotechnological techniques that require special government attention, and 2) ways of responsibly and effectively meeting the need for consumer information. Toxicological aspects should not be included in the advice as this subject will be covered by the Health Council. The Health Council therefore set up the Committee on Toxicological Aspects of Biotechnologically Prepared Products (referred to further as the Toxicological Committee) on the 6th of April 1990. The Toxicological Committee considers both biotechnologically produced medicines and food products. The Biotechnology Committee was set up by the Food Council. Both committees

will deliver identical advice concerning subjects related to food safety evaluation. The advisory report of the Toxicological Committee to the Minister and Parliamentary Secretary of State of the Ministry of Welfare, Health and Cultural Affairs has been published [Health Council, 1992]. The report of the Biotechnology Committee is expected in the near future. Aspects to be covered in this report will be potential applications, consumer aspects, nutritional value and safety, and regulatory and marketing policy.

At present, responsibility for evaluating the safety of novel foods rests with the producer and no testing of this evaluation is done by the government. The Toxicological Committee advises that foods, obtained by means of recombinant-DNA techniques or cell fusion, should be evaluated on the basis of decision trees set up by the Committee and that this evaluation should be assessed by the government at a later stage. The Toxicological Committee not only advises with respect to toxicological aspects but also to product safety in general. *In the report the Committee states that new biotechnological techniques do not inherently cause more safety problems in comparison with traditional breeding, but there is still insufficient data on these techniques.*

To arrive at a safety evaluation of biotechnologically prepared products, the Toxicological Committee has set up four decision trees with relation to:

- single chemicals and chemically defined mixtures,
- food products of vegetable origin,
- food products of animal origin,
- food products and ingredients of microbial origin.

The decision trees are mainly derived from those proposed in the IFBC report with an extra one for products of animal origin.

For the evaluation of products from the first category the starting-points are identical to those of the IFBC decision tree for the same category: sufficient knowledge on the genetic background and toxicity of the components of the producing organism as well as genetic stability and variation in expression. Production under GMP (good manufacturing practice) conditions is demanded in all cases. In contrast to the IFBC report, the Committee does not stipulate the absence of genes which code for antibiotic resistance as a criterion for release onto the market. This is thought to be unnecessary as the final product will be purified, thereby reducing the risk of gene transfer to a negligibly low level. In those cases where the final product contains uncharacterised components, or whenever this cannot be excluded, a 90 day feeding trial is stipulated. Another difference in comparison to the IFBC decision tree is the fact that the latter would re-evaluate the ADI (Acceptable

Daily Intake) of the product if it is likely to be exceeded by consumption of the new product. Dutch regulations only offer this possibility in cases where the ADI is determined wholly or partly by factors that do not relate to the new product.

The criteria as set up by the Toxicological Committee for the safety evaluation of food plants are very similar to those of the IFBC. If the traditional counterpart is a 'GRAS' product, knowledge of the genetic background of the insert and the influence of the insert on the composition of the product is of importance as well as the estimated consumption pattern. In order to detect possible changes in composition of food products of vegetable or animal origin or other macro ingredients, it is deemed necessary to have an analytical reference database. The Dutch Database on Food Ingredients (NEVO) and the Database on Contaminants in Food Products (COBA) located at RIKILT-DLO could serve as a basis for such a database. Marker genes coding for antibiotic resistance when incorporated in a stable way, are deemed admissible.

The most important difference between the Dutch decision tree for food plants and the IFBC counterpart is that the former demands a 90 day feeding trial for each new product. The Dutch decision tree only exempts those products that have the place of insertion characterised to such an extent that it can be established that no detrimental effects to the metabolism occur that may affect the health of the consumer.

The decision tree for food products of animal origin is added because according to the Toxicological Committee sufficient data are available to set up criteria for this category of products. This decision tree evaluates products of genetically modified animals as well as those of animals that have been treated with substances produced using biotechnology. The hazard in the latter category relates to contaminants of the preparation as a result of the production process. The preparation itself, or derived residues, will usually be evaluated according to the Veterinary Medicines Act. In products of animal origin it is equally important to be able to detect possible changes in composition as a result of genetic modification. In principle, the same starting-points apply as to products of vegetable origin. On the other hand it is thought that the production animal will function as a safety filter, for example when administering contaminated preparations. Furthermore, it is deemed unlikely that pleiotropic effects will cause the induction of toxic compounds in those products. The health status of the animal is considered an important criterion in this respect.

With regard to the final category of products, food products and ingredients of microbial origin, it is not always clear whether a product should be considered as part of this or as part of one of the

preceding categories. Mushrooms, for example, as a primary food product, will be evaluated as a food plant, whereas single substances produced by micro-organisms should in general be evaluated according to the second decision tree.

The report stresses the importance of characterising the influence of production conditions on the organism and the strain used in addition to the criteria set up for this category by the IFBC report. It is known that under certain conditions a usually non-pathogenic strain can produce toxins. No genes coding for antibiotic resistance should be present in the organism, because gene transfer to, for instance, the gut flora, and the resulting gene expression, cannot be excluded. The organism should be produced under GMP conditions.

In contrast to the IFBC tree, the Dutch variant stipulates an exact characterisation of the incorporated genetic construct and pays special attention to the effect of the new product on the nutritional status of the consumer. In addition, two extra questions are added so that the use of micro-organisms as probiotics can be assessed. Finally, as the ultimate test, a 90 day feeding trial is demanded whenever insufficient data are available about the place of insertion, in order to guarantee that the genetic modification has not caused any changes in the metabolism which could be detrimental to the consumer.

Safety evaluation of these categories of products should be carried out by, or for, the producer and subsequently tested by an independent institution. This final control is also necessary as the decision trees allow room for manoeuvre. After three years the criteria and the evaluation procedure should be reconsidered.

2.7 EC - DG III Internal Market and Industrial Affairs

The EC Committee has recently accepted a draft proposal by the Directorate General Internal Market and Industrial Affairs (DG III) for the regulation of novel food products and ingredients in the member states in order to prevent the obstruction of free trade traffic as much as possible [EC, 1992]. The proposal relates to all food products and ingredients that have hitherto not been available for large-scale human consumption and to products that have been considerably modified by the production process with respect to composition, nutritional value and/or estimated consumption pattern. Food additives, flavours, extracts and products that have been treated with ionising radiation do not fall under this regulation.

Fundamental principles for releasing novel foods and food ingredients onto the market are:

1. The products should be safe for the consumer when consumed as food in the intended levels of use;
2. The products should not mislead the consumer;
3. The products should not differ from similar foods or food ingredients that they may replace in the diet in such a way that their normal consumption would be nutritionally disadvantageous for the consumer.

Before a new product is released onto the EC market the producer will have to demonstrate that the product complies with the above mentioned criteria by using the results of scientific research carried out on the product. One or more qualified and independent experts (the Committee will set up a list of national experts) will have to confirm the conclusions of this research. Once these conditions have been met the product can be marketed provided the Committee has been notified. This notification should include a summary containing all the information needed to demonstrate the safety of the product. The Committee will subsequently notify the competent authorities in the member states.

If the new product entirely or partly consists of viable organisms or if insufficient scientific data are available to demonstrate the safety of the product then the producer will have to apply for authorisation, as notification alone will not be sufficient.

Where a genetically modified organism is involved, evidence of approval from the competent authority (the Ministry of Housing, Physical Planning and Environment in the Netherlands) for research and development of the organism concerned as well as the complete technical dossier necessary for the risk analysis with regard to population and environment as indicated in the appropriate EC guidelines, should be added to the application. The Scientific Committee for Food will take this dossier into consideration in their evaluation. The Committee will be consulted for each new product falling under this regulation which could possibly affect the health of the consumer.

The Committee will inform the member states of an application for authorisation. The member states can subsequently offer advice and/or supply scientific information on the subject. The Standing Committee for Foodstuffs will deal with the application for authorisation. This Committee will advise on measures proposed by the Committee within a term set by the chairman. This term will be fixed according to the urgency of the matter. The EC Committee will take the advice into consideration.

If a member state opposes the release of a certain product onto the market it is possible to postpone or limit the release of the product in the country concerned. The other member states as well as the Committee should be informed immediately of this measure and of the reasons behind it. The Committee will have these reasons investigated as soon as possible by the Standing Committee for Foodstuffs. The whole procedure will then be repeated. If the Committee decides that the national measure should be modified or cancelled, the procedure will lead to the establishment of appropriate measures.

2.8 OECD - Environment Directorate

In 1990, the OECD set up the Working Group on Food Safety and Biotechnology within the Group of National Experts (GNE) on Safety in Biotechnology. The Working Group deals with scientific issues in the field of safety of new food products and ingredients. The Group focuses its attention on microbial, vegetable and animal products that have been produced using biotechnological techniques. Food additives, contaminants, substances used in industrial processes and packaging materials are not considered. *The Working Group aims to develop scientific concepts to demonstrate whether these new products have the same degree of safety as their traditional counterparts.*

In the introduction of the draft report published in 1991, a historical review was given of a number of aspects that have influenced food safety through the ages [OECD, 1991]. Variation in the composition of nutrients and toxins is considered in the light of breeding processes to increase yield, improve resistance and obtain better sensory characteristics, but also as a consequence of external, environmental conditions and of internal factors such as subsequent ripening stages.

The Working Group has as a starting-point scientific principles that must be used with a certain amount of flexibility, taking into account the specific knowledge of the new product. This knowledge refers to the introduced characteristic, the consequences for the nutritional value and level of toxins, the estimated consumption pattern and the way the product is prepared or processed. The Working Group advocates a case-by-case approach more emphatically in comparison to the other proposed evaluation procedures, and consequently does not propose structured guidelines in the form of decision trees.

It is the opinion of the Working Group that new biotechnological techniques do not require a fundamentally different approach or new safety standards. The basic principle in the evaluation is to determine the substantial equivalence between the new product and its traditional counterpart, if it exists.

To be able to determine substantial equivalence it is necessary to have sufficient data on both the traditional and the new product, as well as on the incorporated genetic construct and its expression products. Other important factors are the method of preparing or processing and the estimated consumption pattern.

The Working Group considers food products or ingredients derived from organisms that have incorporated a well-characterised construct which, with a certain probability, does not code for toxic elements or cause pleiotropic effects, equivalent to their traditional counterpart. Changes in composition or possible pleiotropic effects can be detected by determining the levels of known components such as nutrients and toxins.
Once substantial equivalence is established the product can be evaluated in a similar way to its traditional counterpart. Where no traditional counterpart is available and the concept of substantial equivalence cannot be applied as such, evaluation should be based on experience with comparable substances or products.

The report of the Working Group gives nine examples of the application of the principles described above. The examples vary from a traditional product such as LEAR (Low Erucic Acid Rapeseed) oil to products from transgenic animals. First, for each example, a conceptual survey is given of aspects that may be of importance for the final evaluation. These factors can be rather diverse: according to the concept of continuity (if the product does not change, but the consumption pattern does), the concept of temporality (temporal changes in composition of the traditional product), the concept of variability (variation in toxin levels in the subsequent ripening stages), and the concept of substantial equivalence. Important factors are:

- knowledge of the product
- evaluation of the traditional counterpart. This is highly dependent on the nature of the product.
- the possible availability of a database in order to be able to evaluate the traditional and novel food product. For example, the RIKILT-DLO Database on Contaminants in Food Products is mentioned.
- knowledge of the new factor within the product
- possible additional evaluation procedures, whenever this is considered necessary, with motivation for these procedures.

The approach of the Working Group is innovative in the sense that it focuses on scientific concepts rather than on technical dossiers. The case-by-case approach is absolute. There is no decision tree to guide the evaluation of dossiers or to assist those formulating specific conditions for approval. Furthermore, no specific requirements are described with respect to technical details on the genetic construct and integration process needed for the evaluation.

2.9 United States - Food and Drug Administration

In 1992, the Food and Drug Administration of the United States published a Statement of Policy: Foods derived from new plant varieties [FDA, 1992], thus becoming the first official institution to publish guidelines for the introduction of products from genetically modified plants onto the market. This statement relates to new plant varieties, irrespective of their mode of production, either by classical or advanced breeding techniques. The FDA states that existing legislation is sufficient for the evaluation of products derived from plant varieties obtained by new techniques. *At present new plant varieties are not systematically evaluated for food safety. The FDA is of the opinion that this will not be necessary in the future when new plant varieties obtained by means of advanced techniques will be marketed.* It is stated that the legal status of the food product depends on the characteristics of the product and on how it will be prepared or processed and not on the method of production. Knowledge of the latter can, at the most, be a factor to guarantee adequate evaluation of the final product. *In specific cases the FDA will require a pre-market review. In all cases the producer remains responsible for the safety of the product for the consumer.*

In the United States, the marketing of food additives is regulated under the Food Additives Amendment. For food additives a pre-market safety evaluation is required, unless it is a GRAS substance or can be recognised as such. A limited list of GRAS substances has been published by the FDA. Producers are advised to contact the FDA if they are of the opinion that a certain substance, not listed as GRAS, should be considered to be so.

The FDA states that an inserted gene and its expression product should be considered as a food additive. This implies that in principle a pre-market evaluation should be done, unless it is a GRAS substance or can be recognised as such. Some other aspects of new plant varieties that possibly need to be evaluated in order to be able to determine the food safety of the products are: unforeseen effects, such as pleiotropic effects, possible changes in levels of natural toxins and of important nutrients, possible (increased) allergenicity and the presence of antibiotic resistance genes as selection markers.

Labelling is considered necessary in those cases where the consumer may be mislead. This could be the case, for example, if a new product differs from its traditional counterpart to such an extent that the traditional name no longer relates to the modified product. Labelling will also be required if an allergen has, or has possibly, been introduced into a new product. In general, however, the FDA is of the opinion that labelling of products obtained by advanced breeding techniques is not necessary.

Several decision trees are included in the statement to guide the producer in the evaluation of a new product. Six decision trees have been set up relating to the safety evaluation of the donor plant, the incorporated fragment, the introduced proteins, introduced or modified sugars, introduced or modified fatty acids and a general decision tree combining these different elements. On several points within these decision trees the producer is advised to consult the FDA experts if the status of the product is not clear. The consequence of this, however, is that the final criteria by which the FDA evaluates new products, remain rather vague.

A complicating factor in American legislation is the division of tasks between the different institutions. For example, the responsibility for marketing pesticides rests with the EPA (Environmental Protection Agency). At present, a genetically modified insect or virus resistant plant should be evaluated by the EPA as far as the introduced resistance is concerned. This implies that food safety aspects of the introduced gene and marker genes, if present, should also be evaluated by the EPA.

2.10 Conclusions

In the previous sections, guidelines as proposed (in draft) by different organisations, for the introduction of genetically modified products have been summarised. In some cases these guidelines are part of existing guidelines for products produced by more traditional techniques, in other cases guidelines are proposed as a specific completion of already existing guidelines. All guidelines relate to products obtained by means of a number of specific techniques, especially recombinant-DNA techniques.

All guidelines for the risk evaluation of the introduction of new products are based on a case-by-case approach. This does not mean, however, that there are no major differences between the different approaches. There is considerable variation in the room for manoeuvre between the different guidelines. Some proposals for risk evaluation are based on dividing new products into different categories (especially the IFBC and the Dutch advisory organs). The OECD draft proposal applies the case-by-case principle in the most far-reaching way. In this sense, the more recent proposals tend more towards the case-by-case approach.

The proposal of the Dutch Health and Food Council is very similar to the IFBC proposal: evaluation focuses on the product and the new products are divided into different categories. The report of the Scandinavian advisory committee also tends to evaluate the product and not the process. The products are, however, not divided into categories, at least not at the beginning of the evaluation process. The room for manoeuvre seems to be rather limited with these proposals.

Figure 2 Elements in proposed evaluation strategies.

	A	B	C	D	E	F
IFBC	+	+	-	-	-	+
Skand.NNT	-	+	?	+	+	-
VK-ACNFP	+	+	-	+/-	+	+
FAO/WHO	-	+	_	_	+	?
NL-Health Council	+	+	-	+	_	_
EG	?	+	+	?	?	-
OECD	-	+	-	-	-	?
VS-FDA	+	+	-	-	+	+

A: makes use of decision trees (the nature of these decision trees may differ slightly)

B: case-by-case approach

C: new legislation necessary

D: risk analysis including feeding experiments

E: allergenicity as a factor in risk analysis

F: responsibility of the producer

+/- in specific cases

The British proposal does not divide products into categories. Both products and process are evaluated according to the same scheme. The British decision tree is not a decision tree as such: it can be used as a guide for the producer to find out which data need to be supplied for the safety evaluation. No consequences are attached to these guidelines and the room for manoeuvre in these guidelines is therefore considerable.

Risk evaluation as proposed by the FAO/WHO working group aims at both the product and the process used. The technology used determines the amount and the nature of the information to be provided with relation to the product. Although risk analysis is described per category of new products, the working group advocates generally applicable guidelines on a scientific basis instead of specific guidelines for different product categories. The working group does not make use of a decision tree. The room for manoeuvre is considerable.

The EC guidelines are not guidelines in the same sense as in the other proposals. In the proposal, the evaluation procedure is described and a category of products indicated that, in general, will need to follow the authorisation procedure.

The OECD, as already stated, goes the furthest in applying the case-by-case approach. This approach is more in agreement with that of the FAO/WHO.

The FDA, finally, also applies a case-by-case approach, in which the producer is offered six decision trees to enable him to make a risk evaluation of a new product. In all cases the producer remains responsible for the safety of the new product. If these decision trees do not offer enough information about the legal status of the new product, the producer is advised to consult the FDA. The final criteria of the FDA for the introduction of new products therefore remain rather vague. Special attention is paid to the introduction of antibiotic resistance genes and the possible introduction of potent allergens. The FDA is still studying these subjects. As, in principle, no authorisation procedure is necessary for the introduction of genetically modified products, the FDA advocates a regulation of these products that is fundamentally different from the regulation proposed by the EC and OECD.

As the room for manoeuvre of most proposals is quite considerable, it is not clear to what extent the introduction of these guidelines will result in conformity of evaluations of new products in the different countries. Several organisations have already declared themselves to be in favour of general, international guidelines with relation to novel foods. However, the publication of the EC draft regulation and the FDA guidelines in the United States show that world-wide agreement on the subject will not be reached in the foreseeable future. An important advantage of generally accepted guidelines would be that the introduction of these new products would not have an adverse effect on free trade traffic. It is therefore important for international organisations to take the lead in the discussion on guidelines for products and product ingredients derived from genetically modified organisms.

Risk evaluation of single substances and simple chemical mixtures will not usually lead to major differences in risk evaluation. Also, in relation to the evaluation of genetically modified micro-organisms, no major contrasts can be observed in the different proposals, although gaps in taxonomic knowledge may cause some difficulties in the evaluation [Franklin, 1988].
It can, however, be foreseen that when introducing evaluation strategies for transgenic vegetable and animal products as described in the foregoing sections, there will be major differences in the evaluations. The international organisations in particular advocate evaluation based on molecular and biochemical analysis, comparing the new product with its traditional counterpart. The FDA also advises producers to follow the analytical approach. The Scandinavian and Dutch reports, on the other hand, tend to base the evaluation on toxicity tests of the product.

The reports favouring an analytical approach indicate that the performance of toxicity tests in animals is an elaborate and time-consuming procedure resulting in data that are difficult to interpret. These toxicity tests should therefore only be carried out after ample consideration.
It is not clear whether the chance of insertional mutagenesis, with detrimental effects for food safety, increases with the application of genetic modification compared with traditional breeding techniques. In traditional breeding techniques tissue culture steps are also often part of the procedure. In thirty to forty per cent of the cells this could lead to changes in DNA structure causing morphologically detectable changes [Van den Bulk et al., 1990]. An extra argument against the standard performance of animal tests is the possible variation in genetic composition within the genetically modified variety. The variety to be marketed is often the result of back-crossing a number of successful transformant lines with the unmodified variety. An analytical approach would probably furnish more information on variation in biochemical composition and on possible toxicological consequences than anim
al tests.

3 BOTTLE-NECKS

3.1 Introduction

Whenever a safety evaluation of products from genetically modified organisms is performed, several difficulties may be encountered. These are mainly related to the modification and only in a few cases to the techniques applied. Some of these aspects will be discussed briefly in this chapter.

3.2 Transformation systems.

The different transformation systems that have been developed all aim to transfer genetic information from one organism to another. Even when a gene from the same species is involved, reproduction will usually occur in a micro-organism. The most frequently used transfer system in plants, the Agrobacterium tumefaciens system, also uses a micro-organism as vector.

Although damage to the cell will differ considerably, depending on the system applied, selection on the basis of morphologic characteristics will, on the whole reduce these differences to comparable values. In other words, if a certain transformation system causes major damage to the genome of the cell, changes will soon be morphologically detectable and can therefore be rejected. Other, more subtle, genetic changes as a result of the insertion will be comparable to changes caused by other, more elegant transformation systems. A transformation system will therefore be used once it has proved to be efficient. Future improvements in existing systems, for example by applying homologous recombination can, however, be an important factor with respect to safety evaluation. When homologous recombination is applied, a DNA fragment can be incorporated on a previously determined site in the genome. The chance of insertional mutagenesis will therefore be considerably reduced. There are no techniques yet available to establish homologous recombination in plants.

Specific changes in plants, caused by genetic modification by the Agrobacterium tumefaciens system, cannot be ruled out. Field experiments have shown that various species show a weaker growth pattern independent of the nature of genetic modification. Experiments are currently being performed with original Agrobacterium tumefaciens strains to determine whether this effect also occurs in these strains. Another possible explanation for these observations could be to do with the secondary effects of the generally used marker gene npt II.

3.3 Marker genes.

In order to be able to recognise successful transgenesis experiments it is necessary to introduce a second gene, the marker gene, with the target gene. A gene can function successfully as a marker gene if its expression product is demonstrable in the earliest stage after the experiment. Several genes that result in resistance to a number of external factors, such as antibiotics or herbicides in the medium, meet this requirement. A further advantage of using these compounds is that unsuccessful experiments are directly rejected.

Neomycin phosphotransferase II (npt II) gene (= aminoglycoside (3') phosphotransferase II, APH (3') II) is by far the most applied marker gene in genetic modification experiments. The expression product results in resistance to, amongst others, neomycin and kanamycin. These antibiotics are used in human medicine only on a limited scale, whereas in veterinary medicine they are used extensively, especially in cattle and pig breeding [Bijvoet, 1991]. Aminoglycoside antibiotics are administered for infections caused by anaerobic gram-negative bacteria. Anaerobic bacteria (99% of the gut flora) are naturally resistant to aminoglycosides due to the lack of respiratory quinones and binding sites necessary for the transport of antibiotics to the cytoplasm.
On the subject of the food safety of this gene as a marker gene for the genetic modification of tomatoes, the gene technology company Calgene Inc. has written a rather elaborate report that has been submitted to the FDA for approval. The report is based on theoretical considerations. Practical research in this field is still very limited. This report states that the marker gene could be harmful in three different ways: as a result of direct toxicity, by immunological effects (allergenicity) or by reduced effectivity of medical treatment. Toxicity of the DNA as such is not likely. The report states that no indications have been found of possible transformation of gut epithelial cells *in vivo*. These epithelial cells are completely differentiated and have a half-life of five to eight days. Transformation of these cells will have such a limited effect that treatment with antibiotics will not be endangered. Furthermore, no cases of spontaneous transformation of a bacterium strain in the human GI tract are known or can be found in experiments done under similar conditions. The report describes a risk analysis for such a transformation and draws the conclusion that in a worst case scenario one bacterium of the gut flora will become resistant to neomycin and kanamycin in one out of every thousand people consuming an average daily amount of genetically modified tomatoes. Compared with the huge numbers of aerobic bacteria that are already resistant to these antibiotics this seems to be negligible [Calgene, 1990].

Concerning the effects of the gene product, the Calgene report states that the introduction of resistance genes is not likely to have any immunological effects, because the gene is already present in the gut flora and because mutations in microbial cells cause a constant flux of new protein and nucleic acid molecules. Reduced effectivity of the antibiotic is thought unlikely as the enzyme is rapidly degraded in the GI tract. Furthermore, for effective inactivation of, for example, kanamycin, ATP is necessary. ATP is probably not available in the GI tract [Calgene, 1990].

A method to remove the antibiotic resistance gene from the plant genome after a successful transformation experiment has recently become available. For this purpose an extra gene is introduced which accomplishes the removal of the npt II gene. The extra gene is subsequently removed by cross-breeding [Moffat, 1991]. However, this results in considerable delay in the overall breeding procedures.

In addition to antibiotic resistance genes, herbicide resistance genes are also used as marker genes on a limited scale. Both kinds of genes have the advantage that, together with rapid identification of successful experiments, direct selection can take place. To date only a few examples are known of market-ready products in which a herbicide resistance gene has been incorporated as marker gene. A (toxicological) risk evaluation of these genes and gene products has not yet been performed.
In micro-organisms, scientists have succeeded in developing methods to remove an essential gene, necessary for normal growth and reproduction, from the genome. This gene is subsequently re-introduced into the genome together with the (transgenic) target gene. The gene thus functions as a marker gene. It seems only a matter of time before research will produce similar systems for higher organisms. If no major arguments arise from a toxicological evaluation, it would seem acceptable to allow the use of resistance genes as marker genes in the meantime. At the same time, research to find alternative systems should be stimulated, as well as post-marketing research on the effects that the introduction of these marker genes will have on the antibiotic resistance of the gut flora.

3.4 Regulator elements.

It seems only a matter of time before it will be technically feasible to genetically modify every plant, whether mono or dicotyledonous. The limiting factor for the number of new applications of these techniques will increasingly be the identification and isolation of genes of interest and regulator elements. Modification of a product of secondary metabolism (not a protein) requires considerably more fundamental biochemical knowledge than is currently available [Lindsey et al., 1989]. Progress in research to identify specific regulator elements such as tissue or developmental stage specific

promotors and enhancers is also rather slow. Furthermore, specific activity of a regulator element in one species does not necessarily indicate similar activity in another species. It is, however, possible to manipulate promotor activity by replacing specific sequences within the promotor [Schöffl et al., 1990].

Only a few tissue-specific promotors have been identified. Examples of such promotors are the patatin promotor (expression in the potato tuber), the phaseolin and conglycinin promotor (cotyledon), glutenin and ß-hordein promotors (endosperm) and a α-amylase promotor (aleuron) [Lindsey et al., 1989].
In addition to these specific promotors there are several constitutive and inducible promotors. The constitutive promotors used most often are the nos (nopalin synthase) and CaMV (Cauliflower Mosaic Virus) promotors. The latter, on average, causes a thirty times higher expression rate compared with the former. The inducible promotors identified so far are sensitive to light (promotors of the small subunit of Rubisco and of chlorophyll binding protein), to anaerobiosis (of alcoholdehydrogenase I), to heat shocks (heat shock proteins), to fungal factors (chalconsynthase) or to injuries (potato inhibitor II) [Lindsey et al., 1989].

A group of promotors currently the subject of extensive research are those of the heat shock proteins (hsp). This is a group of proteins that are expressed by a sudden increase in temperature and possibly also under the influence of other stress inductors. At the same time the translation efficiency of other mRNAs is temporarily reduced. This reaction probably protects the cell against the effects of heat stress. Although the hs proteins consist of different families and show considerable diversity, strong similarity can be seen among these proteins in different organisms. The exact function of the hs proteins has not yet been described. They seem to be independent of temperature, which is important for the development of the organism. Within the promotors of the hs proteins, sequences have been found that are important for heat induction and for increase of expression. Knowledge of these elements has enabled scientists to design promotors that can achieve higher expression levels [Schöffl et al., 1990].

It is also possible to increase expression levels dramatically by duplicating promotors and enhancers in transgenic plants. Applications of these new regulator element (target gene) combinations further increase the necessity for research on systems for metabolite profiling and component analysis. As already stated, it is feasible that a promotor causes tissue or developmental stage specific expression in one organism while causing constitutive expression in another. Intermediary scenarios, for example expression in several but not all tissues, are also feasible. Insight into this is important for a proper safety evaluation.

3.5 Component analysis and metabolite profiling.

It has been shown in the past that ongoing selection for disease resistance in classical breeding programmes can lead to unacceptably high levels of natural toxins. One example of this is the high solanin content in resistant potato varieties [Anderson et al., 1987; Mahon, 1990]. Fundamental knowledge about the biochemical background of natural resistance is often lacking. It is therefore important to perform a component analysis on new varieties, produced by either classical or new breeding techniques. In this way, changed levels of natural toxins and important nutrients can be detected. The OECD considers this as one of the most important aspects of risk evaluation and has for this reason introduced the concept of substantial equivalence. This concept is used as a conceptual basis for the risk evaluation of products that only differ in a single or few aspects from a comparable classical product [OECD, 1991].

Figure 3 Basic elements in the risk analysis of genetically modified complex products.

Exposure:
- to newly introduced proteins, including marker proteins
- to the whole complex product

Substantial equivalence:
- to the new product in comparison with the traditional counterpart

[DNA analysis:
- of the place of insertion and flanking regions]

(Immuno)toxicity:
- of the newly introduced proteins, including marker proteins
- of the traditional components, if significantly elevated.

In various reports, including that of the FDA, this concept has been further evaluated and it is stated that the level of natural toxins should not exceed similar levels in other edible varieties of the same species [FDA, 1992; Stewart, 1992]. Other reports, however, indicate that a changed composition with respect to nutrients or natural toxins does not necessarily imply that these products can no longer be considered safe. The preparation process of the product is also important for risk evaluation. If this process aims to eliminate the natural toxins, for example by boiling, then this should be considered in the risk evaluation.

It is generally expected that as a result of genetic modification in plant breeding all new agricultural products, produced by either traditional or advanced biotechnological techniques, will be viewed more critically. Biotechnology can thus contribute to increased consumer awareness. Because of this there is an increasing need for adequate methods of analysing complex products. It is also important that existing databases, in relation to food components, be combined as soon as possible, so that modification analysis of complex products can (for the time being?) concentrate on known nutrients, toxins and antinutritional factors. In this way more insight can be gained into the components of a product, but also into the variation in composition depending, for example, on variety, stage of ripening or sampling location.

So far, little is known about the variation in components of different varieties. In order to be able to assess the increased or decreased toxicity of a new product it will be necessary to gain more insight into biochemical changes that result from a certain genetic modification other than the intended ones. For this purpose databases will need to be set up to supplement existing data with that from specific research projects.

An alternative system for component analysis is metabolite profiling, whereby an image of the new product is obtained using techniques such as NMR, LC(GC)-MS, (N)IR. This image can be compared with that of the classical counterpart of the new product as well as with profiles of products of other varieties. It will not be necessary to identify individual compounds in the spectra: the profile as such will provide information on possible changes in biochemical composition. If in this way large differences between the classical and the new product are found, additional experiments to characterise the biochemical nature of these differences can still be considered. If these differences can only be seen in a single or a small number of transformant strains it will in general not be advisable to continue with such strains. In this way metabolite profiling can be of use early on in the breeding programme.

3.6 Immunotoxicity.

One aspect that is mentioned in several reports and which is considered to be an important factor in risk assessment when introducing a protein into the food chain, is the immunotoxicity of that protein. Immunotoxicity is a relatively new field in experimental research. A protein, or a protein fragment, can cause an immunological effect when a certain epitope is introduced unchanged or when the protein, or fragment, has been modified in such a way that an effect can occur. Indeed, it seems that food intolerance (non-immunological food over- sensitivity) occurs more frequently than

food allergy. Food intolerance may be the result of the lack of specific essential enzymes, but other causes cannot be excluded. The underlying cause of food intolerance is seldom known and few research projects are devoted to the subject [Health Council, 1991]. Now that in the near future a number of new compounds may be introduced into the human diet in considerable amounts, it is time to intensify research on allergenicity and on the induction of intolerance.

Several different specialised immune tissues and cells are located in the GI tract. The most important of these are Peyer's patches (grouped T and B lymphocytes in the small intestine), over which epithelial cells are folded (M cells) that are likely to have a presenting function. Peyer's patches contain precursors for secretory IgA. Other important cell types are the intra-epithelial lymphocytes, the lamina propria lymphocytes, macrophages and mast cells. Testing systems for immunotoxicity are mainly based on changes in the composition of these immune cell populations. A clear description of the different forms of immune response after antigen stimulation can be found in the Health Council report on immunotoxicity [Health Council, 1991]. This report reviews the existing testing systems for determining the immunotoxicity of individual compounds. The strategy proposed in the report to determine the immunotoxicity of (new) compounds is mainly based on the multiphase system proposed in the 1987 IPCS report. Indications for immunotoxicity in the first phase will lead to second phase research with more specific tests. However, validated testing systems to investigate the antigenic characteristics of compounds in relation to food allergy are not available. A testing system considered to be promising is the popliteal lymph node (PLN) assay which is based on a lymphoproliferative reaction in the popliteal lymph node. This test used to predict the ability of compounds to cause contact dermatitis and auto-immune disease has so far produced good results. It is not yet clear, however, whether it can be adjusted to identify food allergens. The main problem in the development of useful testing systems is the considerable variation in sensitivity to sensibilisation and subsequent allergies in individuals [Health Council, 1991; Schou, 1990]. In addition to the testing systems mentioned in the report, post-marketing surveillance and epidemiological research is still advised.

3.7 Post-translation modification.

Post-translation modification could be a factor in both toxicity and immunogenicity. The exact effect of, for example, glycosylation on immunogenicity, receptor recognition, degradation rate and degradation products of a protein, is not known. There are, however, indications that this effect should not be underestimated [Alexander et al., 1984; Goldburg et al., 1990; Mahon, 1990]. Other forms of post-translation modification, such as phosphorylation may in certain cases also cause

toxicological effects. On the other hand the lack of post-translation modification could also cause toxicological effects. The latter may occur if a eukaryotic gene is expressed in a prokaryotic organism, for example chymosin produced by a micro-organism [Mahon, 1990]. More research is required on this subject.

3.8 Labelling

Several reports briefly discuss the labelling of genetically modified products, but they do not always agree on the category of products which should be labelled when marketed. Some advisory committees are of the opinion that all products obtained by genetic modification should be labelled to guarantee the consumer freedom of choice. Other institutions, including the FDA, consider labelling only necessary if a product is modified in such a way that consumption of this product could be harmful to a specific group of consumers. A modification that introduces a known allergen, for example a peanut allergen, into a product not already containing this allergen, would thereby be subject to labelling [FDA, 1992; Anderson et al., 1987].

If labelling becomes obligatory for specific categories of products, analytical methods will be necessary in order to make verification possible. It is clear that good identification methods will be necessary to make effective inspection possible. It is not clear how measures for rapid identification can be taken without introducing an extra risk factor, for example the introduction of an extra identification sequence. Identification will, therefore, need to be product specific. If a cisgenic gene is incorporated, inspection by means of biochemical analyses will not be sufficient and testing systems at DNA level will need to be developed. As every transformant strain is in principle unique, these systems will have to be based on more general characteristics of genetic modification, thereby possibly reducing the sensitivity of the test [Jones, 1992]. Other proposals, such as the introduction of genes that cause marked characteristics to organisms not suitable for consumption, seem less realistic [Anderson et al., 1987].
Another condition for labelling is a good definition of, for example, genetic modification and related terms. It has proved difficult to draw an exact line between different categories of products.

It is clear that informing the consumer is of major importance for acceptance of the new products [De Greef, 1991; Middlekauff, 1990; Hamstra et al., 1989]. In the Netherlands, discussion on this subject is currently in progress. So far it seems that the results are in accordance with the recommendations of the British report: no labelling for products identical to those already in the human diet. A provisional agreement states that the use of microbial chymosin, identical to the chymosin obtained from calves, does not need to be mentioned on the packing.

4 CONCLUSIONS AND RECOMMENDATIONS

The following conclusions can be drawn from the foregoing chapters:

* The proposed guidelines for evaluating the food safety aspects of food products obtained by recombinant-DNA techniques show great similarity, with differences only on minor aspects. Important agreement exists on considering the transgenic expression product to be an additive. Toxicity and exposure levels should be determined. To identify possible pleiotropic effects in complex products, all guidelines apply the principle of comparing the composition of the new product with the composition of its traditional counterpart (substantial equivalence). Differences between the proposed guidelines for complex products are mainly with respect to the necessity for additional tests to determine, for example, toxicity and allergenicity.
  Criteria for approval of a new product are far from clear. To gain more insight into these criteria and to screen guidelines for imperfections, it will be necessary to evaluate several test cases according to a selection of the proposed procedures. The results of these evaluations will be valuable when trying to reach an optimal evaluation strategy. It is proposed to start with evaluating products according to the guidelines proposed by the Dutch Health Council, OECD and FDA.

* The case-by-case approach is generally accepted and seems to be the best one for evaluating the considerable variety of genetic modifications in the various products. Moreover, by applying a case-by-case approach the latest scientific findings can be included in the evaluation, thereby guaranteeing maximum safety of the new products. One disadvantage of the case-by-case approach is that it may require more effort to safeguard uniformity of evaluation. Communication with the consumer could also be affected by this approach and the evaluation procedure may take more time. It is feasible that, once sufficient experience with the evaluation of novel products has been gained, categories of products can be indicated which can be evaluated according to a simpler procedure if knowledge on product and insert is sufficient.

* One discussion point is whether feeding trials with the complex product should be part of the standard toxicological risk evaluation. The more recent reports advocate a more analytical approach in the risk analysis procedure. In view of the problems encountered with animal experiments (difficulties in composing a balanced diet, low safety factors), the choice of an analytical approach appears to be justified. At present only classical feeding experiments are available to test the complex product toxicologically. An alternative to these experiments would in theory be an analytical approach at the DNA (RNA) level or the application of systems such as component analysis or metabolite profiling. However, fundamental knowledge on the functioning mechanisms

at DNA level is largely lacking. Component analysis and metabolite profiling seem to offer enough perspective to obtain an impression of changes in composition after genetic modification in a relatively simple way, while also sparing laboratory animals. If this analytical approach cannot generate sufficient data on the safety of the new product, additional feeding experiments can still be considered.

* There are some consequences if the analytical approach is chosen. Systems to test the concept of substantial equivalence (component analysis and metabolite profiling) will need to be developed and validated. Databases will have to be set up in order to perform risk analyses more efficiently. These databases will need to provide information on the presence and variation of nutrients and antinutritional factors in specific varieties of food plants, including the different varieties.

* In some experimental disciplines additional fundamental research is necessary in order to perform risk evaluations more efficiently. Increased research efforts are especially necessary in the immunotoxicological, gut toxicological and fundamental molecular biological (regulator elements, gene-gene interactions) disciplines. Improved knowledge on plant physiology is also of importance for the safety evaluation of new varieties. Finally, additional knowledge of the phenomenon of post-translation modification would be extremely valuable. In the short-term, research in the fields of post-translation modification, immunotoxicology and gut toxicology of complex products, whether modified or not, needs to be stimulated in order to improve existing evaluation procedures. Moreover, the development of *in vitro* systems could reduce the number of laboratory animals involved.

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TERMS AND ABBREVIATIONS

Antisense RNA: RNA coded for by the complementing, non-coding DNA sequence of a gene. It is thought that this RNA forms a complex with the RNA coded for by the coding DNA sequence of the same gene, thereby inhibiting translation and subsequent expression of the gene.

Cisgenesis: process of genetic modification incorporating a gene of the same species.

DNA (deoxyribonucleic acid): the complex, biochemical substance that genes consist of. DNA contains the genetic information in most living systems. The order of the bases that form part of DNA determines what the expression product of the gene will be, if any.

Electroporation: technique to introduce DNA sequences in a cell as a result of a difference in electrical potential. This difference in potential facilitates the transfer of DNA sequences through the cell wall.

Enhancer: DNA sequence that may enhance the expression of a certain gene in co-operation with the promotor. Enhancer sequences can be located upstream or downstream of the gene or within the gene. The distance between the enhancer and the gene under the influence of this enhancer, can be considerable.

Expression product: a specific protein, polypeptide or RNA molecule, coded for by the DNA sequence of a gene.

Gene: specific DNA sequence, usually coding for a protein that accomplishes a specific function within the organism.

Genetic construct: DNA sequence of a genetic element obtained by applying recombinant-DNA techniques.

Genetic modification: addition, deletion, substitution or other forms of modification of the genetic material of an organism by the application of new techniques, especially recombinant-DNA and cell fusion techniques in a way that would not be possible by natural reproduction or recombination.

Genome: total of genetic material in a cell.

GLP (Good Laboratory Practice): the organisational process and the conditions under which laboratory studies are planned, performed, monitored, recorded and reported.

GMP (Good Manufacturing Practice): the organisational process and the conditions under which products are produced and processed in order to prevent the formation of unintended by-products, and to avoid contamination, mixing or wrong labelling.

GRAS (Generally Regarded As Safe): mark that is given to products or substances that have a long tradition of safe use without being explicitly tested toxicologically.

Homologous recombination: process of DNA-exchange in which sequences in the host genome are replaced by partly identical sequences.

Immunotoxicity: harmful effects of toxic substances to the immunological defence system.

Insertional mutagenesis: mutagenesis resulting from the incorporation of a new DNA sequence.

Marker gene: gene with a recognisable or selectable phenotype which, for this reason, is included in a genetic construct. After the transformation experiment expression of the marker gene will show whether or not the transformation was successful.

mRNA (messenger ribonucleic acid): form of RNA, transcribed from the DNA, that provides ribosomes with the necessary information for the synthesis of a protein.

Mutagenesis: process resulting in the modification of a DNA sequence.

Natural toxin: toxic substance in agricultural food products as a result of biosynthesis in the organism or as a result of natural prevalence in the environment.

Pleiotropic effects: unintended side-effects as a result of a change in the genetic material of an organism.

Promotor:
DNA sequence located upstream of a gene and regulating the expression of this gene. The distance between the promotor and the gene is usually rather constant.

Protocol toxicological research: research to investigate possible harmful effects of substances according to specific research guidelines.

Protoplast: plant cell after removal of the cell wall.

Recombinant-DNA techniques: set of techniques to selectively cut and recombine DNA sequences in order to change the genetic material of an organism in a specific way.

Regulator element: DNA sequence that influences the expression of a gene, for example a promotor or enhancer.

Secondary effects: indirect effects of the expression of a gene on the metabolism of the host organism.

Sensibilisation: increased sensitivity to a substance as a result of an immunological reaction on repeated exposure.

Somaclonal variation: phenotypic expression of genetic changes that can be observed after cell or tissue culture procedures.

Transformation: process to incorporate foreign DNA sequences permanently into the genome of a cell.

Transgenesis: process of genetic modification incorporating a foreign gene into the host genome.

Transposon: DNA segment with the ability to jump within the genome. Under specific conditions transposition can also take place from one genome to another.

Vector: DNA segment used to introduce foreign DNA into the host genome in order to express new genes or to amplify the incorporated gene.