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# Genetic modification of food

**GMO, Genetic Modified Organism, definition**

Genetic Modified Organisms according to the Gentechnikgesetz (GenTG)from 20.06.90 (Genetic Technique Law) in Germany are organisms whose genetical material were modified in a way which is not found in nature under natural conditions of crossbreed or natural recombination. The genetic Modified Organism must be a biological unit which is able to multiply itself or to transmit genetic material.
Examples of modifications covered by this law are DNS recombination techniques in which vector systems are used; techniques by which genetic material prepared outside of the cell is introduced directly in the organism. These techniques include microinjection, macroinjection and micro encapsulation, cell fusion as well as hybridization procedures by which living cells are formed with a new combination of genetic material using methods which are not found under natural conditions.

**Technology of genetic modification**

There are two methods used to introduces a new DNA (gene) in the cell of a plant which is going to be modified:
**The ``shot-gun'' technique:**
This technique is also called biolistic transformation which was developed by Sanford in 1987.
Cereals are not suitable to be modified by transfection with Agrobacterium tumefaciens and the regeneration of plants whose cells walls were enzymic digested is very difficult. A device was built to shoot small particles of gold or tungsten against cells. These particles can be coated with DNA material and are so small that they can penetrate cells without lasting damage.
The machine used gun powder and later compressed helium. The particles are accelerated four times sound velocity.
This method is less labor intensive as the Agrobacterium method. The ADN which is being introduced in the host cell is not so complicated as with Agrobacterium and there is also possible to introduce more then 10 different genes at a time. Biolistic transformation can be used to transform all kind of plants, bacteria, moulds, algae and animals.

**The transfection with Agrobacterium tumefaciens:**[204] This bacterium infects the plant and transfers its DNA to the plant. Agrobacter tumefaciens is a bacterium found in soil. Some strains attack plants transferring a small part or its genetic material to the plant causing tumors.
In the tissue of the tumors Agrobacterium tumefaciens can live and produces there new nutrients (opines).

Opines are products of the condensation of an amino acid and a ketonic acid or an amino acid and a sugars.Examples of opalines are: nopalin(arginine + a-ketoglutaraldehyde ) and octopinOctopin(arginine + sugar piruvate)
Some strains of Agrobacterium tumefaciens have aside of its own ADN genome other plasmides of the size between 200 and 800 kBp (kilobasepairs). These plasmids are responsible for the tumor activity and are therefore called ``Tumor inducing Plasmids'' (Ti-plasmids).

**Ti-plasmides**

They carry genes for:

- Metabolism of opines
- Recognition of wounded cells
- Mobilization and transfer of T-DNA

The T-DNA is the part of the Ti-plasmids which is transfered to the plant ( Transfer-DNA ) and is limited by the 25Bp repetition as Left Border (LB) 0n the left side and the 25Bp repetition as Right Border (RB) on the right side which are the recognition sequence for the T-DNA.
The transfer of the T-DNA takes place only to wounded plant cells. Certain compounds such as Acetosyringon which are released by wounded cells of the plant act as a recognition for the Agrobacterium tumefaciens in order to link to wounded cells. These compounds are found mainly in dicotyledoneans and only few monocotyledoneans such as asparagus. That is why Agrobacterium can be used only in few cases of genetic modification of monocotyledoneans. With addition off syringon even moulds and important monocotyledoneans can be modified by Agrobacterium tumefaciens.
Special techniques made possible to modify the Ti-plasmids and the T-DNA to avoid the production of phytohormones responsible for the tumor activity, the opine synthesis gene were cut out and gene of the resistance to antibiotic Neomycin and Kanamycin was introduced. The binary vector system uses a big plasmid with the vir-region and a small plasmid with the LB and RB.of an Escherichia coli plasmid.
. **Protoplasts transformation:**
Protoplasts are called cells without cell walls.
This method uses pectinase and cellulase enzymes to digest the cell walls of plant tissue.
The vectors used are similar to the method of the shot-gun. The transfer of the DNA to the host is done with the aid of polyethylene glycol or short electrical shocks. This is called electroporation. Selection of the transformed protoplasts an regeneration of the cells is very difficult with this method.
To avoid possible resistance of bacteria to these antibiotics it has been tried to substitute the resistance gene with a gene responsible to the production of isopentenyl transferase which induces a new side shoot of the plant indicating the transformation.

**Selection systems**

Selection of the transformed cells is being done with antibiotics Kanamycin or gentamycin and neomycin . These antibiotics act toxic on cells without transformation.
To avoid poss The DNA material which is being introduced in a plant must contain four parts:

- The gene coding for the new property: For instance be a resistance to dry or excessive wet weather or a resistance to a new chemical agent such as a synthetic herbicide.
- A promoter signal: It is a start signal for the cell to start the activity of the gene such as the production of a specific protein. Most of the promoters which are being used were derived from the Cauliflower Mosaic Virus ( CaMv) called 35S promoter
- A terminator signal: It is a signal for the cell to stop at this point the information concerning the

alien gene. Most of the terminator signals used are derived from Agrobacterium tumefaciens a soil bacterium. It is the nopaline synthase gene (NOS) of Agrobacterium, called terminator NOS or NOS 3´

- A marker gene: Often is a resistance to antibiotics gene used in order to select transformed cells.

**A US definition of GMO** "The term "Genetically Modified Organisms" refers to plants and animals containing genes transferred from other species to produce certain characteristics, such as resistance to certain pests and herbicides." In the European community Genetic Modified Organism and its release to the environment are covered by following rules:
1.- Rule 90/220/EWG from 23.04.1990 concerning the release of Genetic Modified Organism in the environment.
2.- Rule 90/219/EWG from 23.04.1990, modified by the Rule 94/51/EWG concerning the use of GMO in closed systems. 3.- Novel Food Decree concerning foods and ingredients which have not yet been used for human nutrition in a worth mentioning extent before.This includes foods covered by the Rule 90/220/EWG; foods initial prepared by means of Genetic Modified Organism but not containing the initial GMO any more such as sugar obtained from genetic modified sugar beet, foods with modified primer molecular structure, foods which had been prepared with or from microorganism, mushrooms or seaweed, foods which had been prepared with unusual techniques which modify significantly the structure of the food.
The content of the German GenTG is similar to the content of the European Rule 90/220 EWG and Rule 90/219/EWG.
The genetic modification of food has the prime score to produce food with:

longer shelf life,

better properties,

using less insecticides in agriculture.

This is true in case of soybeans but Roundup Ready soybean can be efficiently cultivated only with the insecticide of Monsanto. The worldwide insecticides used for soybean will be monopolized therefore by Monsanto

Efforts are being made to breed cereals with better proteins,rape seed with fatty acids better suitable in case of certain diets, other plants missing proteins causing allergies and lactic acid bacteria resistant to virus in the production of milk and meat products thus turning the process of production and the product itself safer.

Please note that the tomatoes on market in Germany are not the same in taste and structure as they had been for years ago, but they have a longer shelf life as before. This however is not due to genetic modification. It is a result of natural crossing of different types of tomatoes.

One should however consider the loss of quality of fresh tomatoes sacrificed on the effort to commercialize the tomatoes and to insure a long transport and a long shelf life.

Unlike to the experiments of Gregor Johann Mendel( 1822/1884 The monk of the Augustiner Abbey who discovered the Mendelschen rules of heredity) modern genetic modification of food introduces alien genes from one species to another completely different one, such as one or more gens of bacteria to the chromosomes of plants. The modern genetic technology interferes deeply with the natural structure of nature.
Crossing plants do not introduce special parts of DNA like terminator gens, marker genes as done by

extreme genetic modification techniques.

**GMO tomatoes approved by EU Commission**

One sort of genetic modified tomato has been approved by the Food Commission of the EU. AstraZenca plc, London has applied for this sort of tomato which has better properties for processing. It is harmless after cooking which denature its proteins and gens. It is therefore not suitable to be sold to consumers which eat the tomato without cooking.

**Labeling of genetic modified food in Europe**

The labeling of genetic modified food will be made according to European law EG paper 90/220/EWG. It will be made only in food having the following modifications(EG Paper Nr. 1139/98 and 79/112 EWG)

1.-Food containing alien gens, proteins or DNA .

2.-Food on which the new gens were removed by processing technologies or refining but are changed in some parts so that they cannot be compared with food of nature. An example is GMO rape seed oil with modified fatty acid composition.

Suggested labeling:"Produced with genetic modified corn." In the list, of the ingredients it can be cited "genetic modified" when Soya or rape seed oil is being used.

Food having no alien genes left are not labeled as GMO food ( Genetic Modified Food ). Soybean oil is not labeled as GMO food because refinement removes all modified genes.

**Ingredients**

Ingredients are also declaration free no matter how manychanges they have undergone (for instance: soybean lecithin is being used as emulsifier in margarine, chocolate and other products )

GMO chymosin, an enzyme used in the production of cheese needs no declaration.

Enzymes are produced by bacteria whose genetic code had been modified,

These enzymes have great utility in the production of monosaccharides such as glucose syrup starting from corn.

No declaration is necessary because no genes or modifications of the resulting product can be demonstrated.One reason to lower the declaration is that meanwhile 90 % of our food would be involved because they had in some way contact with genetic modification.

In October 1999 The Ministers of the EG States agreed with a papers establishing that up to 1% content of Genetic Modified Food are free of declaration.

Refining soybean oil and processing maize and maize starch in Cornflakes for instance high temperatures are needed modifying or destroying the DNA. The GMO-origin cannot be demonstrated with usual methods. These products, even 100% GMO is free of declaration.That is why great retailers gave a ban on Soybean oil because there is no way to be sure to be GMO-free. GMO corn is being introduced in Europe. The resulting corn oil, corn starch, animal feed for cattle in great amount used as silage is free of declaration.

Unilever has announced to label gen modification on food containing GMO soy and corn.

This would include however only food with genetic modified proteins, products with soybean oil are not included.

**Switzerland**

Switzerland has published a food regulation concerning GMO dated on 1.3.1995 which is mainly

identical to the European regulation also here no declaration of Genetic Modified Food is necessary when tho GMO used in the production of the food and the modified genetic material has been discarded.

Meanwhile Unilever UK, Nestlé UK and Nestlé Italy announces that they are going to produce GMO free.
ADM ( Archer Daniels Midland Co), one of the greatest buyer of Soybeans and corn announces to be able to supply Europe with GMO free raw materials. ADM maintains contact with the America n company DuPontwhich makes contracts with farmers concerning GMO crops. It is obvious that the increased efforts to provide GMO free raw materials do have their influence on the price.
The competition between centers of business such as the Far East competing with dumping prices with the European market and even the competition between European countries itself could only be controlled by suppressing the transport facilities increasing worldwide the price of fuel making global marketing so expensiv that local ecological isolated markets would be able to survive.
A short way between producer and consumer needs a normal shelf life making unnecessary special efforts to increase it. The great supermarkets however need special packing and other special effects.
As it is impossible to turn back these commercial organizations there is no way out of this dilemma.
Be informed what is going on. This is the only way to protect yourself.Look what is happening in the BSE story, the modification of food, the growing importance of contaminants, such as a possible comeback of DDT the powerful insecticide which deposits in food. Remember however that you are guilty of all this mishaps.
It is the constant desire of consumption that speeds up the possibilities of marketing.
You alone can turn back time by living a life concentrated on true values. Try to find pleasure on the simple things of life.Do not be a slave of savor.

**Genetic technique** Are the techniques to promote the transmission of hereditary material between living organism.
Organisms bearing alien genes are called transgene organism (transgene animals,transgene plants or transgene microorganism].There are food and their additives which bear alien genes, other even being genetic modified have lost their alien genes during the manufacturing or refining. The later once are similar to natural products and are therefore free of declaration.
Genetic technique is a very young science:
In 1973 genes where transfered for the first time from one bacterium to another and later on, in 1977 the soil bacterium Agrobacterium tumefaciens was used to transfer alien genes into the cell of plants, or the Bacillus thuringiensis (Bt) has introduced proteins in Novartis Bt 176 maize (bt-Maize) The antisense technique indexAntisense technique was developed in 1990. It suppresses some genes, This was used modifying tomato Flav´r and Sav´r The gene producing ripening enzymes is suppressed and the tomato has a long shelve live
Only in 1996 farming in great scale in USA starts with soy, maize, rape seed and cotton.
The European Union allows farming of BT- maize from Ciba Geyigy/Novartis/Sygenta.on 1997.

| Product | producer | modification or function **Germany** |
|---|---|---|
| Novamyl | Novo Nordisk ( DK) | makes flovera better |
| Europe | | |
| | PGS ( B ) | sterility/herbicide resistant |
| Rapeseed | | |
| Tobacco | Selta/Gauloise (F) | herbicide resistant |
| Salad | Monsanto (USA) | herbicide resistant |
| Corn | Monsanto (USA) | herbicide resistant |

| | | |
|---|---|---|
| **Great Britain** | | |
| Tomato | Calgene (USA) | retarded ripening |
| Chymosin | Gist Brocades (NL) | enzyme for cheese production |
| Tomato pulp | Zeneka (USA) | retarded ripening |
| soybean oil | Monsanto (USA) | herbicide resistant |
| Backery yeast | Gistbrocades (NL) | increased speed of fermentation |
| **Netherlands** | | |
| Xylanase | Primalcol (SF) | better cereal products |
| Xylanase | Quest(Unilever,NL) | better cereal products |
| Novamyl | Novo Nordisk (DK) | better cereal products |
| Amylopectin | starch..Ayebe (F) | additive |
| Rapeseed oil | PGS (B) | herbicide resistant |
| Lipase | Unilever (NL) | production of certain fatty acids |
| **USA** | | |
| Tomato | Calgene (USA) | retarded ripening |
| Tomato | Monsanto (USA) | retarded ripening |
| Tomato | Zeneka (USA) | retarded ripening |
| Tomato | NDAPP (USA) | retarded ripening |
| Cotton | Monsanto (USA) | resistance to insects |
| Soybean | Monsanto (USA) | herbicide resistant |
| Potatoes | Monsanto (USA) | resistance to insects |

**Lecithin**

Lecithin is a natural emulsifier which is present in Egg yolk and in soy beans.
The world production of lecithin from soy beans is around 180.000 mt.For the production of chocolate 25% were used.The rest was used for margarine, all kind of food and last but not least for drugs and tonics.
USA produces about half of the world supply of lecithin.

| Producer | Percent of world supply | GMO |
|---|---|---|
| USA | 50 Percent | GMO positive |
| Europe | | |
| from soy beans of USA | 17 Percent | GMO positive |
| from soy beans of South America | 8 Percent | unknown origin |
| South America | | |
| Argentina | 2 Percent | GMO positive |

| | | |
|---|---|---|
| Brazil | 8 Percent | only small parts are GMO free |
| Asia | 5 Percent | For home consumption, No export |

**The slow death of Soy bean**

In Germany soy oil is not used any more in the production of human food.Only lecithin is still present in margarine as there is no substitute for it in frying margarine.
Great efforts are being made in research to develop mono- and diglycerides compounds with equal frying anti spatter properties. The efforts to get free of soy results from retailer specifications demanding GMO - free , organic food in order to respond to the desire of the customer which is greatly concerned with the natural origin of food.
The latest great fears of BSE, Dioxins in Food and Genetic modified food have created a loss of confidence on the public food control departments.
In the case of BSE or dioxins no modification the genetic code of cattle or hens are made. The harm caused to the involved species by these problems are turned back as soon as the cause or the epidemie is overcome.
Unfortunately this is not so with Genetic Modified Organisms such as Soy beans.
The change of the genetic code of the plant spreads out through the species.
In few years there will be no natural seed any more present a turn back will be impossible. All benefits of Soy bean such as oil, lecithin,vegetable proteins. soy milk, tofu and a variety of ingredients and foods made from soy bean get lost once for ever in their originality. The aversion to GMO soy bean is strongly present in Germany, UK, France, Australia and many other countries , sometimes hidden by other concerns. Slowly the use of soy bean is dying.
Monsanto is responsible for a loss of confidence in one of the most important agricultural export article of US. It is of main concern of public life to demand that 1.- Soybean planting, transportation, storage, shipping and processing is handled separately from GMO-free Soy bean to restore confidence on the origin of food.
2.- Make a collection of samples of seeds of Soy bean GMO - free in order to guarantee access of future generation to the original seeds. These measures are of urgent importance as the spread of the GMO seed is being forced all over the world by just one profit minded organization damaging the image and the security of an important part of US resources.
These measures should also be extended to corn as seeds from Ciba (Bt-corn, bT= bacillus thuringensis) [172], later overtaken by Novartis are deeply modified in their genetic codes.

**Soybean as protein supply for animal feed**

[205] Soybean meal which is left from the production of oil is by far the most important ingredient for animal feed. In 1999 the European Union used as animal feed:Million
Soybean meal 26,5 million tons
Sunflower meal 9,1 million tons
Rapeseed meal 5,6 million tons
Pea meal 5,4 million tons
Animal meal 2,4 million tons
Fish meal 0,9 million tons

Animal meal can therefore be substituted by soybean or sunflower. Safety could be increased. The costs of the ban of animal meal as animal feed is insignificant comparing with tho costs to win the battle against BSE.
Soybean meal is not possible to be substituted by other plants. It is therefore important to protect soy

plant from genetic modification which might later on turn out to toxic for the plant.

**Identity preserved (IP) non-GM soy bean lecithin and maize**

The desire across Europe to avoid genetically modified Foods has lead to an increase of demand for organic and GM-free foods.Austria is the most developed market in Europe. 8.6% of its total farmland is dedicated to organic farming.
Germany and France is expected to develop the greatest market for organic and non-GM foods.
The growing demand of non-GM ingredients for industry creates the need for a certified supply of raw materials. Some bodies which certify organic food chains are Skal Skalin the Netherlands, Naturland in Germany Ecocert in France.
Non-Gm soy bean lecithin is now available with Identity Preserved (IP) non-GM certification coming from the state of Parana,south of Brazil. Non-GM colours such as beta carotene, curcumin, lutein, beetroot and caramelised sugar.
Efforts are being made to create xanthan gum derived from GM-free crops of sugar to replace maize derivates because of doubts about the non-Gm status.
Sources of Non-GM, organic, Halal and Kosher status of food ingredients are listed in the Ingrid Database from Fi Data Services, Milton Keynes, UK.
**Some of the improvements of food for the future**:

**Gluten free cereals**: Gen engineering may produce wheat free of gluten. This aminoacid is not tolerated by sprue patients ( a kind of severe allergy ).

**Rice with high level of vitamin A**:In Asia there are regions with undersupply of vitamin A. New seed of GMO rice is rich on provitamin A helping to overcome the deficit.
**Transgenetic oil of rape seed**: The GMO oil of rape seed has a modified composition of fatty acids being more valuable then normal oil.

**Chymosin**: Is an enzyme obtained from the stomach of calf. It is used in the production of cheese.Because of the prevention of cruelty to animals and because of hygienic aspects many people prefer cheese made with chymosin produced by transgenetic bacteria.

**Phytase**: Is an enzyme used in vegetable food for poultry and pig diets. It liberates the phosphor which is bounded as indigestible phytate. Using phytase the amount of mineral phosphor being added to the food may be reduced and consequently the amount of phosphor which is deposited on the fields.
This turns out to be positive for environment reducing overfertilizing with phosphor. Phytase is produced by GMO bacteria and represents the good side o genetic modification of food.
Other compounds which are produced using genetic modified technologies are: Erythropoietin used in the drug Recormon to treat anemias.
rPA ( recombining plasminogenic activator) used in the drug Rapilysin to treatsever cardiac-infarct.
Monocloned antibodies are also transgenetic biochemical compounds with great chances in future
Other positive genetical products may come soon:
Genetic modified organism like Bacillus subtilis producing enzymes which hydrolyses starch in the production of glucose which is the basis of the production of citric acid and other products.
GMO microorganism will soon produce vitamins such as B1 and B2 as well as aromas and aminoacids such as flavor enhancer and aspartame.
Allergies: It is to believe that the number of allergies is not increasing with the genetic technologies. The real cause of an increasing risk of allergies is due to a contact to local unknown proteins resulting from globalization of the food marketing by exotic food being imported from everywhere. A recent example is the kiwi allergy and other causes such as environment.

GMO soybean: On the fields of America and Brazil the main producer of soy oil is the seed of " Roundup Ready soybean"being developed by Monsanto.
This seed is tolerant to the herbicide " Roundup ", also manufactured by Monsanto.

The GMO soybean is authorized to be sold in Europe to be used as food for animals and the oil for human food.

In Germany, due to the activity of Green Peace soybean oil is not being used for food.

GMO corn: The GMO corn (called B.t.corn) developed by Ciba-Geigy was authorized to be sold in Europe on the 4.2.1997. The modified corn bears the following gens :
1.- A gene for the production of a B.t.-toxin which protects the plant against a specific insect.
2.- A gene called pat'-gene for the tolerance to the herbicide "Basta " containing Phophosphinotricin. This gene was introduced only to select th plant with the B.t-toxin gene.
3.- A gen called amp'-gene which was introduced in the plant together with the B.t.-toxin gene. It is the ampicillin resistance gene.
The amp' gene produces TEM-1 beta-lactamase which is the most common beta-lactamase found and is responsible for the resistance to ampicillin from 50% of all Escherichia isolated today, from, which 90% are being caused by the RTEM1 type.
The gene is plasmid coded and is denominated as amp' or bla(Tem-1) and exist on a series of cloning vectors such as pBR 322-derivate and pUC- series.
TEM-1 has a low activity against new cephalosporin and may be inhibited by beta-lactamase blocker such as clavulane acid or tazobactame. However under certain conditions there may be created a resistance to amoxicillin / tazobactame as well other combinations of beta-lactame/beta lactamase - inhibitors.
Recently an increase of mutation of TEM-1 and SHV-1 beta-lactamase is noted producing in some cases resistance to new cephalosporin and monobactame.
These derivates are labeled as "extended-spectrum-beta-lactamases" (ESBL), These enzymes were found in Klebsiella pneumoniae, Escherichia coli, Serratia marcescens and other Enterobacteriaceae.
Mutation of TEM1 beta-lactamase such as TEM-30 to TEM-41 may be the reason why the inhibition through clavulane acid is very low.
Bush 1995 has introduced therefore a subclass labeled as "2 br" for these variants.
The "inhibitor resistant TEM-beta-lactamases" ( IRT) was found only in Escherichia coli and in certain Proteus mirabilis and Klebsiella pneumoniae.It might happen that IRT also spreads to Haemophilus influenza and Neisseria gonorrhoeae having frequently TEM-1-beta lactamase.
The use of ampicillin to treat Enterococcus and Haemophilus influenzae infections should be made nowaday preceded by a sensibility test and using suitable beta-lactamase blocker by necessity.
The possibility of a transfer of gens from B.t. corn from Ciba to bacteria cannot be completely excluded.

To transfer the amp'gene to a bacterium it is necessary that the plant releases the amp' gene together with the "origin of replication" (ori) from pUC without damage. The plant cell usually degrades DNA during release with his own nucleases.
DNA enzymes in the paunch and the digestive tract from mammals and in the soil where bacteria may grow also destroy the gens.
The ability of bacteria to take over alien gens is seldom. Only very few bacteria have this ability, together nucleases found everywhere the probability of a transfer of the amp'gene from corn to a bacterium is very low.
The formation of a replicon in the cell, as for example the binding of the extremities of a DNA - fragment could only happen through an illegitimate cross over.
The host place of the origin of a replication exists only in a very limited number of Enterobacteriaceae.
All the above mentioned restrictions make the possibility of a transfer of amp' gene from plant to a

bacterium seem to be unlikely.
It is supposed that all human beings have an amp' gene bearing Escherichia coli in their intestinal tract without having been exposed to beta lactame antibiotics. Approximately 50% of all clinically isolated Escherichia coli are already resistant to ampicillin of which 90% have a TEM-1 beta-lactamase.
The amp' gene already being so frequent there is no further danger to increase significantly the number of resistance by a transfer of the gene from Ciba corn to a bacterium.

However the alien gene has no function in the new corn. In future developments it is important to avoid marker gens with resistance to antibiotic or herbicides.

**Detection of GMO in food**

There are many genetic modified foods on market. To supervise the declaration new methods of analysis were needed which are based on molecular biological principles.
Detection can be made looking for the new specific protein or detecting the new genetic material.
The detection of GMO is very difficult because there are so many other compounds which may interfere in the detection, such as polysaccharides which can inhibit the polymerase chain reaction leading to false negative results.
The food processing causes a denaturation of the proteins on the DNA which is being on test being responsible for failing to be recognized by primers and antibodies. The tests should therefore be suited for the specific processing methodes used. The genetic modified material is often present in very small amounts. Sometimes the transgenetic protein is located in other parts of the plant and the part which is being used as food has no or very little transgenetic material such as the Bt toxin which is present in leafs but not in maize kernels of Novartis BT 176 maize. Usual methodes of GC-MS, HPLC and capillary electrophoresis are unable to detect them. Immunological detection of the transgenetic proteins such as Western Blot or ELISA are now used.
The most effective method to determine transgenetic material is to amplify the alien sequence of the promoter and the gene.
The analytical methods contain the following steps[37]:
1.- Extraction of DNA:It is necessary to extract the genetic material free from other impurities which might interfere in further steps of the analysis.
.- PCR reaction ( Polymerase Chain Reaction)
The PCR reactions are suited to multiply and amplify specific fragments of DNA that are alien genes to the food being

analysed. The primer starter molecules used in the beginning of the reaction decides which sequence of DNA will be multiplied. To avoid false negative results due to inhibit action of impurities during extraction of the DNA it is important to include a positive reaction.
3.-Making the PCR product visible
Through gelelectrophoresis (agarosegelelectrophoresis). The products of the PCR reaction can be made visible together with the determination of the length of the base pair, the alien gen.
4.-Confirmation of the results
The confirmation of the results are being made by controlling the sequence of the base in the PCR product using specific sequence restriction, hybridization with specific sonde
Nested PCR and
Sequencing
The basic PCR gives only qualitative indications. To obtain quantitative results the Competitive PCR or the RT-PCR should be used.

**Competitive PCR**

Is not so expensive as RT-PCR but there are dilutions to be made which take much time. If two sequences are present with the same complementary DNA sequence for the primers to annel they compete for binding of the primers. A DNA sequence ( internal standard) which is much shorter as the target DNA The amplification products can be separated on a agarose gel. The fluorescence of these products is proportional to the amount of amplified DNA. There are two bands on the agarose gel. Dilutions must be done until the brightness of both bands are equal.

**RT-PCR Real-time PCR**

The amount of molecules produced during each stage is measured rather then at the end as happens with competitive PCR.
To demonstrate the Presence of modified DNA the PCR-Method is today favored. Other Methods are the gelelectrophoresis sequencer and ELISA .
These methods are used as well in food chemistry as well in clinical researches because they are based on the same principles.

**Western Blot**

The method of Western Blot the extraction of the transgenetic protein from the food is done by means of a nitro-cellulose membrane which binds the proteins. The membrane is immersed in a solution of a specific antibody together with an enzyme resulting in a colour reaction. This method is very labour intensive and therefore not being used in routine.

**ELISA (Enzyme Linked Immuno-Sorbent Assay**

It is based on the same principles used for Western Blot. The membrane is substituted by a plastic plate with 100 and more wells being therefore suitable for many tests at the same time.

**Round Up Ready Soybean,Monsanto Co**

The Round Up Ready Soybean is a glyphosateresistant soybean (Glycine max). Glyphosate inhibits the enzyme of the metabolism of aromatic aminoacids in plants, the so called EPSPS 5-EnolPyruvylShikrimi-3-Phosphate-Synthesis.
The gene of glyphosate tolerance comes from Agrobacterium tumefaciens, strain CP4.
The transferpeptid as carrier of the EPSPS in the chloroplast comes from Petunia hybrid (transit-signal-sequence)
The detection of GMO Soybean Round Up Ready is made using the specific primer pair B1/B2 which couples with the promoter sequence CaMV 35S and with the transit - signal- sequence,
This primer pair gives a PCR-product of 172 Bp which hybridize with the DNA- sonde H-35s-ar1 after the transfer to the membrane [36].The range of options of PCR analysis of genetically modified organisms (GMOs) is expanding from day to day. As there is a great variety of commercialized GM plants grown in USA and in Europe, being exported all over the world , laboratory work is getting always harder to detect all possible GMOs as reference materials are not always attainable.

**Risks of genetic engineering**

: According to a statement of Prof Wolfgang van Daelen, WZB, Berlin 1997 today there are no empirical or plausible theoretical arguments that genetic modified food represent a greater risk to the consumer as it is with normal food.
Risks can never be completely eliminated, however there are no real risks known until present date.
Future research to avoid risks of genetic engineering will be handled in two ways:

Proactive risk research: This way asks what can happen ?

Monitoring: The supervision by experts of the fields and the processing of food.

The greatest security in genetic engineering is hoped to be attained with use of both ways.

**Allergy and soybean**

[35] Why such a trouble about genetic modified soybean ?
Soybean was the first genetically modified plant to be introduced in widespread agriculture.The approval of the new plant by the Food and Drug admSoy inistration in USA (FDA) is made by determining the allergenic potentials of the plant.
For this purpose the FDA has established in 1992 a guideline to determine the allergenic potential of a plant.
In 1995 followed the guideline of OECD and WHO to determine allergic components in plants.This guideline contains the principal ideas of the guideline of 1992.These guidelines have three main points:

- The origin of the new gene.
  Some origins are already known as allergenic such as Brazil nuts, peanuts, milk, kiwi, rice, corn, spices, eggs, meat, fish, crustaceans and many pollen. Being no allergic components of the origin known the identity with other known allergens has to be verified.The stability to digestion, the stability during processing the final amount in food and the identity to other non allergenic proteins must be determined.
  Being there no allergenity found in these tests the new plant can be approved.
  Being allergenity of the origin known the following test have to be made: In vitro RAST/ELISA being negative Skin Prick Test in vivo are made.
  When these tests are negative the object can be tested as food and in case of showing no negative actions it can be approved.
  In case of a positive reaction in the RAST or ELISA test as well in the Skin Prick Test but no allergenity detected in the resulting food can be approved with a warning about the possible allergic reaction must be given on the label.
  In case of the Round Up Ready-Soy bean allergenic tests of the origin cannot be performed because the alien gene has his origin in soil bacteria and there are no antibodies of the blood of allergic persons available.

  During development of the modified soybean there were genes of the Brazil nut used.Antibodies from persons allergic to Brazil nut indicated allergenity of the new plant.
  A warning on the label turned out to be necessary. The further research on this plant was therefore abandoned.
  This was an example of allergenity of the origin gene being taken over by the new host.

- Host of the new gene.
  Some hosts are already known as allergenic such as soybeans. Allergic reactions to normal soybean are known. GMO Round Up Ready Soybean had therefore be tested on regard of possible

allergenity taking over from his primary host.
The immunoblotting method shows the forming pattern of the serological antibodies resulting from the new soya proteins.

The Round Up Soybean reacted identical to the normal unmodified soybean.In relation to allergenity the GMO soybean is therefore identical with the normal plant.

- The allergenic activity of the new protein There exist no antibodies for the new proteins. Direct test is not possible.
  The evaluation of the toxicity is made with the following considerations which are common to all allergic proteins:
  The proteins must be stable against the stomach acid an to enzymes like trypsin and pepsin in order to trigger an immunological reaction.

The alien gene from GMO Soybean produces the enzyme CP4 EPSP-Synthase.This enzyme was compared with existing allergens.

**Size of the molecule**

Only the size of the molecule was identical to known allergens.This is however not relevant because the molecule is inactivated by heat.Processed Soybean meal is therefore not allergenic because of this heat barrier.

**Digestion**

The CP4EPSP-Synthase molecule is not resistant to digestion, being destroyed in the stomach within 15 seconds.

**Bounding to long chained sugars**

Typical allergens are bound to long chained sugars , the new enzyme does not have sugar in his molecule.

**Degree of strangeness**

The degree of strangeness is an important element of comparison with other allergens.The new enzyme CP4EPSP-Synthase is similar to EPSP - Proteins from baker yeast and indexBacillus subtilis Bacillus subtilis.Both of them are considered as GRAS (Generally Regarded as Safe)

**Amount of Allergen in the final food**

The amount of CP4 EPSP-Synthase in the final food is so small that only 0,001% in total and 0,08% in the proteins of the food are found. Allergenic substances are usually the major part of the food.

**GMO soybean oil and allergenity**

There are no allergic reactions known from refined oil of GMO soybean.The modified allergenic residues which are left after refining are below 1 ppm.
CP4EPSP protein and Round Up Ready Soybean is therefore cnsidered as not allergenic.

**French position to GMO**

[76] Some environmental organization like Agir pour l'environnement, Greenpeace, France nature environment combatte the GMO.
Some organizations like the FNPL (Federation Nationalle des Producteurs de Legumes) and the CP (Confederation Paysanne) are against transgene food.
These Organizations are united to fight health- and environment- risks. According to them everything being resistant to antibiotic should be forbidden. Other Organizations like the FNSEA and the CNJA support GMO when:

1. Possible health- and environment risks are eliminated,
2. An acceptable solution concerning labeling of GMO food is found,
3. The national agriculture and the distribution system of food is protected against distortion of trade.

**Recall of GMO maize seeds in France**

In the end of April 1999 there was a recall of maize seeds in France (Elsace) after being proved that the seeds were genetically modified.The recall was confirmed by Pioneer Seeds company.In Germany there were also US GMO maize seed found. DER BUND (German environment organization) says that it should be avoided that frontiers between genetic modified food and natural grown food are erased. The consumer should keep the right to choose between both foods.[149]
Only Germany and Spain have authorized the use of genetic modified maize. All other member s of the EU do not accept it.

**PTI: More unmodified Soya proteins**

Protein Technologies International (PTI) most important producer of soya proteins, garantee that it can supply the demands of genetic unmodified proteins from soybeans in Germany. According to PTI it is a fairytale that US soybeans are not separated in GMO and unmodified. To make sure no modified GMO is being used PTI has developed an Identity Preservation Program which is similar to the "Cert ID" scheme for unmodified foods recently introduced by the trade companies (April 1999)[].

**US exporters want to supply demands of GMO-free Soybeans and cereals**

The exporter and cereal specialist Northland Seed and Grains in Minnesota garantee its products to be 100% GMO-free (April 1999)[151].
Two great cereal buyers accept only grains which can also be sold in Europe.

**Unilever UK :Renunciation to genetic modified ingredients**

Van den Berg Foods UK and Birds Eye Wall's (part of Unilever UK) will stop the use of GMO ingredients changing the with alternative unmodified ingredients, what also includes GMO-free Soy products (April1999). The efforts to produce GMO-free products show that there is a growing market for these products.
Meanwhile Monsanto introduces for autumn 1999 the GMO Roundup Ready Soybean in Brazil
The industry likes the"Brave New World" from Aldous Huxley.Rhone-Poulenc,a chemical giant and Limagrains producer of seeds united to create the Rhobio Company being responsible for biotechnology activities.
It has even been tried to change "Geneticaly modified " to " Geneticaly improved ".
The Nestlé Group refers to rising prices on GMO-free products.

The food dealers like Carrefour, Casino, and Systeme U make sure to get garantee as GMO-free from the producer.It has shown that it is almost impossible togarantee GMO-free products because basic components of food are already modified.Carrefour sells products with GMO-free garantee. The Danone company , Paris announces not to use GMO - raw materials in their production in Europe.
The dealers are afraid that customers guided by dubious publications can make their own buying decisions to get out of control.
The dealers support therefore the labeling of GMO. Other supermarket chains in Great Britain which took out of their shelves products with genetic modified ingredients including meat from animals which had been feed with GMO animal feed around July 1999: Sainsbury, Safeway, Marks and Spencer, Northern Foods, Unilever and Nestlé GB as well as Nestlé Italy. Sainsbury searches for GMO-free cereals for animal feed to produce GMO-free meat and poultry [162].

**Terminator-Gen**

In the EU there are rules demanding farmers to pay licenses to seed breeders when seeds from the last crop are held back to be reused once again. The seed breeders say that they are loosing much licenses in Austria where these rules are still not introduced.
USA breeders are therefore working to introduce a new gene in their seeds, the so called "Terminator Gene" which lets seeds germinate only one time.There is no use to keep seeds for the next time. Farmers have to by their seeds from the breeder. If the terminator gene spreads out in free nature all plants will stop to germinate. The use of genetic techniques should not be allowed to collect fees or other financial purposes.[167]
Monsanto has made its golden rice available free of charge to developing countries. This rice had been modified with genetic engineering being enriched with vitamin A. The rice could prevent millions of cases of blindness caused by vitamin A deficiency. Monsanto hopes to sell the specific agro chemicals for this plant.

**Genetic modified potatoes**

[134] Dr. Arpad Pusztai from the Rowett Institute in Scotland responsible scientist liberated the news that rats being fed with GMO potatoesinjured in growth and in its immune system.
The results were declared as misunderstanding and Dr. Pusztai was sent into retirement.
A snowdrop-gene had been introduced in the genetical material of the potato. This alien gene should keep worms and insects away. Further research carried out by Dr. Pusztai made clear that there was a potential menace to animals and mankind. Damage of internal organs,malfunction of the immune system and alteration of growth and were demonstrated by rats being fed with the genetic modified potato.
At the moment it is not clear from where the toxicity comes. Some scientists find the promoter-gene to be responsible for it.
The same promoter-gene is being used in the Roundup Ready Soybean.
If this proves to be true all food having soybean derivates from genetic modified soybeans should be considered as harmful to health.

**French retailer against genetic modified food**

[134] The French retailer Carrefour, Paris announces to withdraw all genetic modified foods.About one third of their own trade-marked articles are affected.
Carrefour is not an enemy of genetic technology, but it seems impossible to predict the results of genetic modification on foods for long terms.Therefore the retailer wants to go the safe way avoiding genetic modified products.
According to Carrefour[134] GMO labeling EU directive proves to be insufficient to provide protection

or information for the consumer as to many exceptions are allowed.
Seven important European retailer enterprises have founded a consortium against genetic modified foods. This consortium embraces Migros (Swiss), Carrefour (France), Sainsbury(Great Britain, Marks and Spencer, Delhaize Le Lion (Belgium), Esselunga (Italy) and Superquina (Ireland).
The aim of this alliance is to clean own trade marks from genetic modified ingredients (vwd/1.4.99/12/mi.

**GMO-labeling in USA**

US-citizens claim for GMO-labeling in a petition which has been given to the Congress as there are no rules from FDA.

**Herbicides at the base of imidazolinon**

[134] Genetic modified rapeseed resistant to the herbicide imidazolinon is already on market.
The pharmaceutical enterprise Cyanamid Canada and the University of Saskatchewan of Canada develops imidazolinon tolerant wheat. The used gene had been detected in winter wheat.

**Celiac disease**

[135] Celiac disease is caused by allergy to gliadin, an aminoacid from gluten of wheat, rye and barley. Gluten is present in flour of theses grains and is therefore present in all farinaceous foods as well as traces in starch of these plants. Starch is an ingredient of a long chain of industrial products.The German Ministry of Research ( BMF Bundesforschungsministerium ) coordinates researches to eliminate the genes responsible celiac causing components of the gluten.
Unfortunately industry does not help the project as there is no financial profit expected.Monsanto hopes to release a glyphosate tolerant wheat type in 2002.
Imidazolinon resistant sugar beets were already developed and are on test.

## Cultivation of genetic modified seeds in Brazil

In September 1999 the cultivation of genetic modified soybean[146]will start.
Fields in the state of Rio Grande do Sul and the south of Mato Grosso and Goias will be cultivated with Roundup Ready soybeans from Monsanto over the Monsoy enterprise.The seed was released by the Brazilian commission CTNbio (Commissao Técnica Nacional de Bioseguranca).
Consumer organizations and government sectors have made resistance against GMO soybean. The GMO plant is coming over the border from Argentina.
Hoechst Schering has received the authorization to cultivate herbicide tolerant rice in Brazil.
According to CNTBio (Brazilian biotechnology safety commission) the worldwide culture of GM plants is 28 millions hectares
Soybeans are represented with 40% followed by maize, tobacco, cotton, tomatoes and potatoes.
USA has 8,5 millions hectares of GM plantations, China 1,8 millions hectares, Argentina 1,4 millions hectares and Canada 1,3 millions hectares.
Du Pont de Nemours and Co has entered the Brazilian market with Du Pont do Brasil with its head in Sao Paulo taking over Pioneer Hi-Bred international Inc in march 99.
As Du Pont has overtaken Sementes Dois Marcos the way is open to the production of genetic modified soy bean seeds together with modified wheat seeds.
The first product of Du Pont in Brazil will be a modified maize seed which was modified without gene transfer so there is no formality necessary to release the seed.This maize has a very high content of oil. (vwd/6.4.99/mi)

**GMO and worldwide famine, an interview with Ian Wilmut**

Can genetic techniques solve the problem of famine in the world?
Ian Wilmut, genetic specialist who cloned the sheep "Dolly" said in an interview with the Brazilian magazine veja (4.11.1998,page 14):
"It would be exaggerated to say so. The actual production of foods is more than sufficient to feed the whole planet. The trouble is that the food is unequal distributed. Instead of producing more food we have to improve the storage of already existing food and we have to distribute them in a fair way. It is sad to know that people still die of hunger just because we do not find a political and social model which enables us to distribute food in a way with more justice. The solution of the famine is political and not scientific. What genetics can do is to give a contribution that this comes true."

**Patents on GMO-plants**

The DBV (Deutscher Bauernverband)German Farmer Union has urged the German Government not to take over the European Biopatent guideline in the German Patent law. The DBV says that European Commission opens the possibility of o wide spreadüread patent on plants.It is also possible that patent protected gens spread in nature contaminating other plants Farmers would be than unwillingly colliding with patent claims. According to the German Farmer Union are plants and animals a part of natural life basics and should not be withdraw from general availability. Plants and animals are part of nature and should not be put under the monopoly of certain business groups.

**GMO rapeseed in Europe**

GMO-rapeseed has been released in Europe by Advanta Seeds without knowledge of the farmer. The GMO-seed was distributed in large scale in Germany,France and Sweden and England for two years. France and Sweden gave order to destroy the GMO- fields. German Environment Ministry says that there is no regulation to force the destruction of the German fields as there is no direct menace to public heath. This is a sign of inexcusable weakness of the German Environment Ministry which is unable to protect purity of nature. Advanta Seeds says that it was a mistake but it is supposed to be a strategy of the seed companies to contaminate rape seeds with GMOs in order to avoid the GMO-free alternative to GMO-Soya which is being refused by retailers in Europe.
The contamination of nature is getting ahead because logistics, flying pollen, handling during processing in storage, on ships and trucks.

**GMO Sugar beets in Germany**

According to daily news from 10.10.2000 sugar beet plants were genetically modified and not allowed released by KWS SAAT AG in Einbeck (Kreis Northeim) Germany. They are resistant to the herbicide Round up. KWS SAAT AG says that it was a technical mistake which caused the release. This is the proof that genetic technology is being handled careless. It also may be a part of a strategy to contaminate nature in order to avoid the GMO-free alternative. It is possible that the gene passes over to weed turning these plants resistant to herbicides.

To counter the growing contamination of nature with GMOs the European retailers urge the producers to guarantee GMO-free production. Even the 1% contamination which is granted as unavoidable

contamination with GMO in connection with GMO-free labeling is not allowed in their products.

**Recall of Aventis GMO maize ``StarLink''**

Aventis has started a recall of its GMO maize ``Starlink'' according to press release from 12.10.99 due to pressure from US- Agriculture Ministry and the Environment Protection Agency ( EPA ).
StarLink maize had been liberated for animal feed. This GMO maize had been sold by farmers to the Azteca Mill in Texas. The maize meal produced from StarLink seed was sold to Kraft as ingredient to the Taco-Bell foods.
Half of the crop of StarLink was sold to the farmers of Iowa.In Europe StarLink was mingled with non-GM seeds. Now almost all plantations are potentially contaminated with StarLink.
Starlink Maize contains a protein which is supposed to cause allergic reactions on humans.
Some Japanese importers denied to import StarLink-Maize as animal feedstuff to avoid the possibility of being used for human food.
Aventis Crop-Science says there will be no sells on seeds of Starlink in 20001.

**Monarch butterfly and the Bt maize**

Many reports affirmed that the Monarch butterfly was killed by toxic compounds of the Bt maize which has parts of the genes of Bacillus thuringiensis. According to the Environmental Protection Agency (EPA) reviews of scientific informations indicates that there are very little risks for the butterfly. Some authors even predict benefits to the butterfly from farming of corn, cotton and potato Bt plants.
John Obycki and Laura Hansen of the Iowa State University had demonstrated that larvae of monarch butterflies grew more slowly and suffered a higher mortality rate when being fed milkweed leaves with deposition of pollen from insect pest tolerant GM maize as sole food source to monarch larvae, causing 20% mortality in larvae within 48 hours with one variety of GM maize. The Environmental Protection Agency trying to reach decision regarding renewal of registrations for several Bt products argues that the situation is different from that prevalent in natural environment.
The larvae of the monarch butterfly feed on milkweed plants mostly in June whereas the peak time of maize pollen shed is from mid-July to August.This allegation however is insignificant as toxic materials for living beings are present bt maize is therefor harmful for nature.

**Nitrogen-fixing bacterium's genome**

The complete genetic sequence of the nitrogen fixing bacteria Sinorhizobium meliloti[215] has been publishhed. Nitrogen is essential for the growth of plants. It can be supplied in form of:

1. Nitrogen fertilisers such as ammonium ions ($NH_4^-$) which is 30% of the total amount needed

2. Atmospheric nitrogen are built through various natural processes reprsymbioseesenting 40% of the total amount needed by the plants. 3. Atmospheric nitrogen obtained by reduction caused by symbiosis between legumes and bacteria known as Rhizobium, reducing atmospheric nitrogen into ammonium ions which can be used for the synthesis of proteins and nucleic acids.