

GM food safety: consequences from improved risk assessment concepts and research results.

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Summary:

GM foods started to come to European markets as a consequence of improved new European regulations. Discussions around the use of GM organisms will continue but need to become more focused on identified problems such as preservation of diversity as the basis for food security and adequacy of uses, realising often highly diverse local agro-ecological situations. Whereas the safety of the GM foods which are presently on the international markets seems to be established, international harmonisation of concepts of risk assessment, risk management and risk communication, based on CODEX principles is mandatory due to an already globalized distribution of foods. International organisations such as the WHO have concluded that problems around GM food production have to be seen in a holistic way, integrating food safety and food security aspects, as well as socio- economic, patenting and ethic issues. While these different aspects of the discussions should not be mixed up the need to tackle all aspects adequately should be respected.

Plant breeding and GM technology:

When new foods are developed some of the existing characteristics of foods can be altered unintentionally, effecting the expression of constitutive components. Unintended effects of traditional breeding methods on levels of anti-nutritional or toxic constituents in food organisms have been characterised in conventional organisms. Organisms derived from breeding methods which include tissue cultures may have a somewhat enhanced possibility for genetic (and epigenetic) instabilities, such as the activity of mobile elements and gene-silencing effects [1, 2, 3]. These effects could result in an increased possibility of pleiotropic unintended effects e.g. increased or decreased expression of constituents or possibly modifications in expressed proteins as well as the interaction of the inserted gene with other genes.

It has been argued that random insertion of genes in GMOs may cause genetic and phenotypic instabilities [4], but as yet no final scientific evidence for such effects is available to support this view. However, single-copy T DNA inserts are known to trigger large scale chromosomal rearrangements, including translocations [5] where portions of the DNA of the flanking region at the T DNA insert site may be duplicated or translocated [6]. T- DNA is widely used as a mutagenic agent to study the role of specific genes and agrobacterial enhancer sequences which are known to drive expression of genes that are proximal to the insertion site (used for activation tagging [7]) are used in GM plants. Environmental factors (e.g. drought or heat) can significantly alter gene expression in both conventional and GM crops [8].

Unintended effects can be divided into insertional effects, related to the place of insertion of the transgenic fragment, and secondary effects, related to the nature of the expression products of the introduced genes.

A WHO/FAO expert consultation in 2003 [9] acknowledged that introduction of a transgene into a recipient organism is yet not a precisely controlled process, and can result in a variety of outcomes regarding integration, expression and stability of the transgene in the host [9, 10, 11, 12].

The desired outcome generally is stable integration of a single copy of the transgene into a single location in the genome, and not in a functional gene or a regulatory element. However, other outcomes are frequently observed, including integration of multiple copies of the transgene at one locus or insertion of the transgene at multiple locations in the genome. Insertion of the transgene into a host gene may turn the host gene off, sometimes affecting the viability or health of the host. Insertion of a transgene sometimes can affect expression of another gene(s). A transgene may become rearranged before integration, thereby becoming non-functional. During the process of transgenesis, undesired DNA sequences may become inserted into the genome, such as marker genes or selectable markers from the expression vector or contaminating bacterial DNA left over from vector production. Hazards stemming from insertional events or genetic instability can be identified by screening and managed by culling individuals that have undesired events during the course of development of the transgenic line.

Expression of the transgene ideally should have no undesired effects on the expression of other host genes or health of the host. Other outcomes, however, have been observed. The transgene can be silenced by methylation or through other mechanisms. Because expression of the transgene often is controlled by novel regulatory elements outside of the host's normal homeostatic feedback mechanisms, expression of the transgene can have pleiotropic effects, upon multiple traits of the host. The use of viral and transposon vectors poses the hazard that the transgene might subsequently move within the genome.

Many of the unintended effects discussed as potential consequences of the introduction of transgenes into organisms have also been seen in foods already derived from organisms developed by conventional breeding methods [13], or methods like the introduction of unspecific mutagenesis by irradiation or chemicals or tissue cultures.

Development of risk assessment concepts of GM organisms

When new foods (crop varieties, animal breeds or microbes) are developed by traditional breeding methods they are usually not subject to specific pre- or post- market risk or hazard assessment by national authorities or through international standards.

Early regulatory risk assessment requirements, such as European regulations (1990) mainly focused on problems for human health and the environment as a consequence of accidental escape of recombinant DNA or GMOs. International regulatory systems covering GM food safety and environmental safety became effective in 2003. The Codex Alimentarius Commission adopted the Principles for the Risk Analysis of Foods Derived from Modern Biotechnology, and the Draft Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants and Microorganisms [14]. These guidelines are based on conclusions of expert consultations where the latest consultation on GM animals updated realized hazards and risk assessment concepts considerably [15].

The concept, that a comparison of a final product with one having an acceptable standard of safety provides an important element of a safety assessment (WHO 1991). This principle was elaborated by FAO, WHO and OECD in the early 1990s and referred to as substantial equivalence. Following harsh criticism of the principle, e.g. misuses of comparisons of constituents [8] or massive interactions of environmental influences on constituents [16, 17], the use of the principle as a risk assessment or as part of formal decisions for notification was changed. FAO/WHO consultation acknowledged that the concept of the substantial equivalence contributes to the design of safety assessment, but was concerned that it would come at the end [10, 11, 16, 17,]. A consideration of compositional changes is not the sole basis for determination of safety and safety can only be determined when the results of all aspects under comparison are integrated. More recently the concept has evolved to a Comparative Safety Assessment [9, 18] where this concept is also the central element of the guidelines for a safety assessment of GM foods of the European food safety agency EFSA.

For an assessment of plants derived from conventional breeding using genetically modified parental lines carrying different traits or resistance genes (gene stacking) recently experts on an US- FIFRA/SAP consultation agreed on the need of review of stacked products where the risk assessment needs to compare evidences from the parental lines as well as from the resulted product [19]. A molecular characterisation of the new product should show that the recombinant sequences in the new product are identical to the insertion /traits in their parental lines and stability of the product needs to be established.

For the assessment of crop plants with deliberately changed compositions, such as for functional foods, the use of the comparative approach needs to be adapted [19].

The Comparative Safety Assessment

The CSA is basically a two-tiered approach. The initial step is comprised of a thorough comparison with the closely related conventional counterpart to identify any differences that may have safety implications for the consumer. This comparison includes both phenotypic characteristics as well as a compositional analysis. The phenotypic analysis should also include comparative health parameters. The compositional analysis will focus on key substances in the animal products under scrutiny and will be subject to changes according to latest scientific state-of-the-art.

The second step of the CSA comprises the toxicological and nutritional evaluation of the identified differences between the GMO and its comparator. As a result of this second step additional testing may be required and can result in an iterative process in order to obtain all relevant information for the final risk characterisation.

In the initial step the information that should be considered includes:

- the transformation process of the genetic modification, including the sequence of the inserted material before and after the transformation event,
- the copy number and site(s) of insertion,
- sequence analysis of the site(s) of insertion, i.e. flanking regions
- stability of the integration (multiple generations),
- the safety of any newly expressed proteins, including assessment of allergenicity,
- occurrence and implications of unintended effects,
- the role of the new GM animal food in the diet and

- the potential influence of processing or spoilage on the new GM food product.

Hazard identification and molecular characterisation:

Hazard identification and characterization are typically the first steps in any risk assessment and an extensive molecular characterisation of the inserted genetic material construct is required, both before and after the insertional event. Following unexpected events in the regions of integration sites in products e.g. soja before intensive discussions in the expert committees detailed requirements for the molecular characterisation especially the sequence and bioinformatics based analysis of the flanking regions to exclude expression of unintended or altered proteins.

Integrated toxicological evaluation:

The safety of the gene product must be assessed on a case-by-case basis. Depending on the knowledge of the expressed product the assessment may range from a limited evaluation process of the available data on the protein, such as amino acid sequence and expression rates in different tissues, to extensive toxicity testing including animal studies. Following the phase of hazard identification, characterization and food intake assessment an integrated toxicological evaluation will combine all the information with relation to the food safety of the complex GM animal-derived food product.

Risk characterisation consisting of a toxicological and nutritional evaluation:

In the case of GMO-derived food the many facets of the CSA including the food intake assessment would need to be combined together. The baseline for the safety of novel food products derived from GMOs, including GM animals, in all cases will have to be the assessment that the novel GM animal-derived food products is at least as safe as its traditional counterpart. If any questions remain after the initial CSA with respect to the safety of the GM animal-derived food products additional tests may be required.

Toxicology studies of whole foods and unintended effects

Toxicology including animal studies need to differentiate between the testing of introduced proteins where usually recombinant proteins are tested in a dose dependent way after assessing homology between the recombinant protein and the protein expressed in the GM organism. However, it will not be possible to test complex GM food products by classical toxicological animal studies in the way they are routinely used to test single compounds. If the genetic modification would result in alterations in an endogenous protein product or metabolite, the traditional toxicological approach would require the concentration of the product to be elevated in the laboratory animal's diet to the extent that the diet will often become unbalanced. This might result in toxicological observations that are unrelated to the product under investigation. The interpretation of such effects has caused irritations e.g. in different point of views of the EFSA and the French expert committee CGB in the review of a BT maize [20]. In the European research project SAFOTEST [21] European research groups elaborated improvements for toxicology testing and concluded on the need to further develop genomic and metabolomic tools for the detection of potential unintended effects. They also differentiated the need of testing whole foods in toxicological studies using rodents (e.g. 90 d) for testing of new proteins in their natural food environment and testing of whole foods to detect potential unintended effects. For testing protein toxicology

spiking of the diet with recombinant proteins might be useful to achieve dose dependent responses.

Horizontal gene transfer (HGT)

The discussion of a horizontal transfer of recombinant genetic material to microorganism was boosted by findings showing a more prolonged stability of DNA than expected in specific situations [22]. Requirements for a natural transformation of DNA to bacteria involve active uptake of extracellular DNA by bacteria in a status of competence [23, 24] or the possibility of rare illegitimate recombination events [25]. Natural genetic transformation was shown in different conditions e.g. in food stuff [26].

The potential for a horizontal transfer of the gene construct has been investigated intensively: Food-ingested foreign DNA may not be completely degraded in the gastrointestinal tract of mice and pigs. Especially research in the EC research project GMOBILITY indicates that small amounts of fragments of DNA from GM plants can be found in different parts of the GI tract [27, 28, 29, 30, 31]. But whereas in all parts of the GI tract transformation of fragment should be possible as evidenced by in- vitro experiments no transformation of marker genes of GM plants to bacteria in the GI tract could be observed in in-vivo experiments even including sensitive models such as gnotobiotic rats. However, FAO/WHO expert panels suggest for food safety assessment that it is prudent to assume that DNA fragments may survive the human gastrointestinal tract and be absorbed by either the gut microflora or somatic cells lining the intestinal tract [9]. Experts agree that possible detrimental consequences of a transfer of recombinant DNA needs to be assessed by taking into account a number of factors including, but not limited to, the specific characteristics encoded by the DNA sequences, the characteristics of the receiving organism and the selective conditions of the local environment of the receiving organisms. Assessment of the safety of genetic construct should include marker genes, where the safety assessment should consider information on the role of the antibiotic in human and veterinary medical uses. The expert panels encouraged the use of recombinant gene sequences without antibiotic resistance genes, or sequences, which could stimulate such transfer and advocated avoiding the use of any unnecessary DNA sequences including marker genes in the genetic construct [32].

Recent public discussions mostly center around problems of GM plants derived sequences detected in different products e.g. milk where these contaminations may mostly derive from contaminations and not from transfer events [33]. However, a careful risk assessment of specific elements of the GMO is demanded by many experts: Inadvertent introduction of constructs containing viral sequences not only has the potential for creating unintended genetic damage but can also contribute by recombination to the generation of novel infectious viruses[9]. A well known example is the generation of a replication-competent murine leukemia virus (MLV) during the growth of a vector containing a globin gene [34]. Some scientists have pointed to the present methodological limitations of a comprehensive scientific evaluation of a transfer to bacteria, mainly because of estimations that only 3-10 percent of naturally existing bacteria in the GI tract can be cultured and therefore be analyzed..

Consensus on HGT from GM plants to bacteria appears to be that it has generally an extremely low probability of occurring, and very much related to the genes, constructs and organisms in question. But as potential consequences of an HGT may be significant the risk assessment of probability, pathways and consequences needs to be a mandatory part of a case-by-case risk assessment. In contrast, the transfer of DNA from bacteria to bacteria, including

mechanisms of conjugation has been seen in the GI tract and needs to be an important issue in the risk assessments for GM microorganisms

Immune responses and allergenicity

Allergic reactions to traditional foods are well known. Major food allergens are allergenic proteins in and derived from egg, fish, milk, peanuts, shellfish, including crustacea and mollusks (e.g., clams, mussels and oysters), soy, tree nuts and wheat. Whereas groups of main allergens are well known and advanced testing methods have been elaborated traditionally developed foods are not generally tested for allergenicity before market introduction.

For GM foods FAO/WHO expert panels focussing on allergenicity aspects [35] have established risk assessment protocols for GM foods where the recipient genes derive from foods with a long history of safe use as well as for genetic modification of organism where the transferred genes derive from organisms with no safe history of food usage. The transfer of genes from known allergenic foods is discouraged unless it can be demonstrated that the protein product of the transferred gene is not allergenic. An example of a transfer of a gene encoding an allergen to a feed crop is the case of a genetically modified soybean containing a 2S-Albumin gene from Brazil nut. As Brazil nut is a known allergenic foodstuff, it was decided to assess the allergenic properties of the transgenic soybean at an early stage of R&D. It appeared that sera from Brazil nut allergic patients could cross-react with the transgenic soybean [36]. For this reason a commercial product was never pursued.

Elements of the risk assessment include the comparison of sequences of the transferred genes (in combination with flanking regions of the insertion sites) with sequence motives from data banks of allergenic proteins, the evaluation of the stability of the expressed new proteins against digestion as well as animal- and immune tests, as appropriate.

Absence of sequence similarity with allergenic protein epitopes and low stability under acidic or proteolytic conditions does not give absolute proof that a given protein is not an allergen. Examples are known which contradict the general rules e.g. where small modifications of a protein sequence determine allergenicity [37]. Allergenicity prediction using protein sequence motifs identified from allergen database has been proposed as a new and superior strategy for identifying potential allergens [38, 39]. Discussions for the use of different minimal motif lengths and consequences of false positive or false negative results continue. Some experts consider that the use of sera from polysensitized patients is important for the testing of allergenicity [40].

The cellular basis of immune responses is not completely understood and a better understanding of the interaction of the immune system and foods in general is required in order to decipher whether specific GM foods may bring with them impacts on the immune system apart from allergenicity. The impact of cell mediated reactions (without involvement of IGE antibodies) on hypersensitivity reactions elicited by foods is a matter of present research [41].

The assessment of GM micro-organisms (GMMs) used as or in foods should include an analysis of potential immune- stimulatory or immune-modulatory effects of the microorganisms in case of a colonisation of the GI tract [10].

Effect of GMOs on human health mediated through environmental impact

Work on environmental health indicators [43] suggests that various agricultural practices have direct and indirect effects on human health and development. Hazards can take many forms, wholly natural in origin or derived from human activities and interventions. The need to assess indirect effects of the use of GMOs in food production has been emphasized by many countries. Potential environmental health hazards of releases of GMOs in the environment have been discussed in a report by WHO/ANPA where health effects have been analysed “as an integrating index of ecological and social sustainability” [44]. For example, the production of chemicals or enzymes from contained GM micro-organisms (e.g. chemicals, pharmaceuticals or food additives), have contributed significantly to decreases in the amount of energy use, toxic and solid wastes in the environment, thereby significantly supporting human health [45].

A further example of beneficial human environmental outcomes of the use of GM crops is the reduction in the use, environmental contamination and human exposure to pesticides demonstrated in specific situations. This has been demonstrated especially through the use of pest resistant Bt cotton in China where decrease pesticide uses and pesticide poisoning of farm workers [46] could be shown in areas where agro-ecological characters show a very high pressure of pests and needs for pesticides.

Out-crossing of GM plants with conventional crops or wild relatives, as well as the contamination of conventional crops with GM material, can have an indirect effect on food safety and food security by contamination of genetic resources. Introgression of transgenic DNA into traditional land races of maize in Mexico with unknown consequences for food safety and security was confirmed recently [46, 47, 48, 50] and has been widely discussed: Both out-crossing and contamination characteristics are dependent on the pollination and distribution characteristics of pollen and seeds of the specific plant. The appearance of Starlink maize, not approved for food use, in maize products used for food in the US, has demonstrated the problem of contamination and highlighted the potential for unintended impacts on human health and safety.

The likelihood of GMO entering and persisting in the environment will vary among taxa, production systems, modified traits, and receiving environments. Therefore e.g. only uses of salmon, which has been sterilized using validated methods in specific plots is encouraged [9].

Improved molecular methods for a containment of the transgenes as well as farm management measures e.g. isolation distances, buffer zones pollen barriers, control of volunteer plants, crop rotation and planting arrangements for different flowering periods and monitoring during cultivation, harvest, storing, transport and processing are under discussion [51, 52, 53] and are important objectives in present research programs.

Conclusions:

The discussions of direct and indirect hazards of GM organisms have triggered intensive scientific research and efforts to harmonise risk assessment procedures for GM foods internationally. Experiences from countries where GM foods have been already consumed for many years and experiences from research indicate no direct principle food safety problems with the GM foods presently on international markets. However, intensive improvements in present bottlenecks for GM foods, the problem of random insertion of constructs resulting in

potential unintended effects and the problem of missing possibilities to contain dispersal of viable materials from GM organisms are necessary if GM technology wants to become an usual method in conventional agriculture. An US EPA-FIFRA consultation therefore encouraged international cooperative developments in site specific insertion methods.

With the generation of more differentiated breeding objectives involving complex traits and gene constructs the risk assessment methods will need to be further developed. In certain areas, especially in the assessment of allergenicity, predictability for safety will be incomplete and it can not be fully excluded that products, from GM- as well as conventional breeding methods may come to markets where established monitoring strategies may be necessary for their removal. However, safety assessment procedures of GM organisms have been developed to an extraordinary high standard where this standard would also be desired for testing of conventional foods.

In consequence, discussions of GM foods in the future should not only centre around food safety risks. Although these discussions may raise the public interest best possible identified problematic developments in the area of modern agriculture need to be addressed : The loss of biodiversity in wild and food relevant species as a consequence of all modern breeding technologies which can improve only limited amount of lines could threaten the basis for food security considerably (FAO, 2003). Methods which allow an in situ propagation of local races or landraces are highly demanded. Another problematic development can be seen in a globalised trading of foods where local specificities of crops (such as a prohibition of use in centers of origin) can not be respected by trading. Crops with specific characteristics should only be used in adequate agro-ecological situations. For BT cotton health and ecological usefulness has been clearly demonstrated in areas with high pest pressure. A use of BT plants in areas without specific pest pressures should not be acceptable but could result as a consequence of international trading. An increasingly pronounced argumentation for more possibilities for local trade restrictions under WTO, especially the SPS agreement is demanded , where under different situations liberalisation of trade may also be economically adequate.

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