Introduction

The study describes and compares regulations of and procedures in toxicological and allergological risk assessment of GM food in the EU and the USA as well as guidelines from e.g. OECD and FAO/WHO. Due to its major role in risk assessment regulations and guidelines pertaining the concept of substantial equivalence are also included. A summary will be provided what is known so far about how the concept is applied. In order to trace the regulatory practice in the US summaries of US applications are also taken into account. The study is part of the project "Toxicology and Allergology of GMO Products" which also included both investigations on the practice of risk assessment on genetically modified plants according to the Novel Food Regulation and recommendations for further standardizing of risk assessments.

Toxicological and allergological aspects are probably the most eminent part in the assessment of potential health risks of food derived from genetically modified organisms (GMOs). Proteins introduced via gene transfer might themselves have toxic or anti-nutritive properties – in case that they are still present in the final food product. In addition, the modified plant itself could exhibit toxic properties. As a result of non-intended secondary effects of the genetic modification, plant toxins might be expressed at a higher rate or so far unknown toxins might be synthesized. E.g. the synthesis of toxic compounds or anti-nutrients could be changed as a result of both increased enzyme activity and enzyme inhibition.

The toxicity of proteins can in principle be assessed by standard toxicity testing. In order to test for secondary effects in a genetically modified plant (GMP), however, these methods are of rather limited applicability. In the former a single compound, in the latter a highly complex mixture comprising a large number of compounds has to be assessed. Since absolute toxicity cannot not be assessed with whole plants or food, a relative toxicity is estimated by application of indirect approaches and by comparison to a conventional counterpart (food or plant).

Proteins could also exhibit allergic properties. Proteins originating from organisms which are known to cause allergic reactions or from organisms with no preceding exposure in food might be introduced into a food stuff. Similar to what is mentioned above allergic properties could probably appear as result of secondary effects, e. g. in case of upregulation of an plant allergen.
Regulatory context in the EU

In order to introduce food originating from GMO (GM food) into the EU market the product has to be approved in the course of an authorization procedure – presently (status: March 2003) according to Regulation 258/97 concerning novel foods and novel food ingredients (Novel Food Regulation). This procedure also includes an assessment of potential health risks. Toxicological and allergological aspects are not mentioned in the Regulation, but are implicitly a focus of the assessment.

Commission Recommendation 97/618/EC was issued to give further guidance for applicants and authorities as well. Toxicological and allergological aspects are thereby put into more concrete terms. However, the Recommendation does not specify in sufficient detail the requirements for data and testing, nor the criteria that should be used in the assessment of the data. As a matter of the legal status of the Recommendation, applicants are not obliged to comply. Hence, the requirements are being put into concrete terms in the course of the authorization procedure, only.

The extent of toxicological and nutritional testing of GM food largely depends on the degree of substantial equivalence. Instead of passing through the authorization procedure according to Article 4 GM food deemed to be substantial equivalent can be introduced by a simplified notification procedure according to Article 5. The concept of substantial equivalence therefore plays a major role in risk assessment.

According to the EU Proposal for a Regulation on genetically modified food and feed this distinction will be abandoned in favour of a consistent authorization procedure. Nevertheless, the concept of substantial equivalence will still be part of the new Regulation. Applications will no longer be submitted to national competent authorities but to the newly established European Food Safety Authority. This should result in a more consistent assessment practice. However, the Proposal does not specify any details on toxicological or allergological risk assessment.

Beyond regulations that are specifically focusing on products resulting from genetic engineering techniques harmonised regulations are covering the placing on the market of seeds, propagating material and new plant varieties. However, these regulations do not consider toxicological and allergological aspects. Conventional food does not need to be authorized or notified at all. Excepted from this general principle are e. g. food additives. The Austrian Food Law considers toxicological aspects only in case of mycotoxins, residues and flavours. As a consequence, toxicological and allergological risk assessment of GM food is essentially confined to GMO regulations.

Assessment of toxic properties

The assessment procedure is based on the concept of substantial equivalence. Only in case the product is not deemed substantial equivalent chemical structure, physico-chemical properties, composition of the food, donor organism, and exposure – especially of particularly vulnerable groups – has to be considered.
In case of total substantial equivalence no further toxicity testing is considered necessary. In case of partial substantial equivalence, further testing is confined to the novel traits of the GMO. The EU Recommendation also includes flow sheets in order to guide the assessment procedures.

OECD and FAO/WHO guidelines specify some more details. Considering some major aspects of risk assessment these guidelines are largely in accordance with the EU Recommendation: the use of substantial equivalence, the need to consider toxicity of both the newly introduced protein and – regarding to known plant toxins – the whole GMP; different schemes of both cultivation and manufacturing should be considered as well as the possible exposure of particularly vulnerable groups.

The most distinct differences can be found in the requirements for analysis of the newly introduced gene products: toxicokinetics, chronic and sub-chronic toxicity, carcinogenicity, teratogenicity are suggested in the EU Recommendation. The FAO/WHO guideline, in contrast, is suggesting these tests for novel non-protein substances only. FAO/WHO proposes sequence comparisons to known toxins and anti-nutrients, thermostability and digestibility studies. Furthermore, evidence should be provided that the gene product investigated (mostly of microbial origin) is structurally and functionally identical to the one which will eventually be consumed (of plant origin).

The EU-Recommendation and the OECD and FAO/WHO guidelines agree that potential toxic properties of the whole plant should be investigated. It is however, stressed that conventional feeding studies are of limited value. Still, the EU Recommendations suggest to carry out at least a 90-day feeding study. Alternative methods are proposed in the Recommendation and in all guidelines.

Assessment of potential allergenic properties of GM food

The assessment of allergenic properties is based on decision tree approaches. Firstly, it is usually investigated, whether or not the donor organism is known to be allergenic. According to the EU Recommendation In-vitro and in-vivo tests (RAST, ELISA, immunoblotting) should be applied if the donor is known to be allergenic. If the donor organism is not known to be allergenic the potential allergic properties should be investigated by comparison to known allergens, considering molecular weight, glycosylation, sequence homology, and stability in the gastrointestinal tract.

OECD, FAO/WHO, and the International Food Biotechnology Council IFBC/ILSI issued guidelines as well. The OECD recommendations propose extended comparisons to known allergens: apart from amino acid sequence comparisons also secondary and tertiary structure should be taken into account. The localisation of homologous sequences should also be considered. The validity of in-vitro digestibility studies is however questioned.

Some of these criticisms are taken into account in a subsequent FAO/WHO decision tree. Comparative studies of functional and structural characteristics to known allergens are recommended. In case there is no homology whatever, the protein should be subsequently tested by serum screening. In doing so the selection of sera is crucial and possible posttranslational glycosylation has to be considered as well. More detailed guidance is given on the testing in order to ensure a more con-
sistent and verifiable procedure. Proteins originating from donors not known to be allergenic should be subjected to homology studies and a progressive serum screening as well as extended digestibility studies. Proteins considered as novel and exceeding a homology of 35% or more than six subsequent amino acids respectively should be classified as allergenic and subjected to direct in-vitro and in-vivo testing.

**Substantial equivalence**

The idea to compare GM food or GMP to conventional counterparts in order to estimate their "substantial equivalence" was introduced into the context of risk assessment in a FAO/WHO expert consultation in 1990. Ever since the concept of substantial equivalence has been developed further in the framework of OECD, FAO/WHO, Codex Committee, competent authorities, and scientific committees. Since 1993 the concept has been established as a basis for risk assessment of novel food and food ingredients in many countries such as EU, USA and Canada.

Comparing GMP and conventional counterparts is based on morphological and agronomical characteristics as well as a set of plant compounds. For reasons of feasibility the range of compounds investigated is however, limited to macro nutrients, known anti-nutrients, and plant toxins. Grave doubts have been raised on the actual safety of a GM food if safety is concluded on the basis of such a comparative analysis if no toxicity and allergenicity testing is performed at all or if toxicity testing was not deemed sufficient and no tests are going to be performed. Furthermore, secondary effects would hardly be detected by this approach anyway. Some critics, therefore, call for long-term whole-food feeding studies while others emphasise the limited validity of these test in this context. Others demand clinical studies in order to properly assess the allergenic properties.

Studies on how the concept is applied in the course of risk assessment procedures reveal both lacking validity and conclusiveness in the line of reasoning and criticise also the limited range of compounds analysed. Furthermore, a lack of consistency in the range of testing and methods applied as well as in statistical evaluation could be shown. This points to the wide margins of interpretation of the requirements.

This could also be seen in terms of selecting the conventional counterpart. Usually a variety that is as isogenic to the GMP as possible and cultivated under identical conditions should be used for comparison. The extent of genetic homology needed is however still a matter of dispute as well as the particular consequences if significant differences will be detected. It is also still not clarified what stage of the food manufacturing process would be relevant for the comparative analysis. So far, the unprocessed plant or the respective part of the plant is used in practice.

As a response to this criticism more detailed recommendations in terms of cultivation and methods applicable for statistical evaluation were given and OECD Consensus Documents specifying sets of plant-specific compounds to be analysed were issued. Databases are going to be established compiling data on the ranges of plant-specific compounds. Besides, novel methods are being tested which will probably be more appropriate to detect any secondary effects and which might prove to be very useful in the course of risk assessment of second generation GMPs. By the use of such methods profiles of complex mixtures of compounds
e. g. mRNA, proteins, metabolites can be visualised and subjected to further comparative analysis between the GMP and conventional counterparts. However, these methods are still not applicable in routine testing and the scientific basis is still to be developed in order to properly interpret any detected differences.

**Risk assessment in the USA**

In the USA genetic engineering is not considered to pose higher risks compared to conventional breeding methods. Hence, there is no need to introduce regulations specifically dealing with products derived from these techniques. Upon application the US Department of Agriculture could assign a non-regulated status which is a prerequisite for commercial cultivation. This largely corresponds to an approval according to Part C of Directive 90/220/EEC (2001/18/EC). GM food is regulated by the Food and Drug Administration (FDA) and in case of pest tolerant crops also by the Environmental Protection Agency (EPA).

In case of GMP containing plant-pesticides the assessment of potential toxic properties is carried out by EPA first. This assessment comprises sequence comparisons to known toxins, in-vitro digestibility studies, characterisation of the mechanism of the plant-pesticide, and testing of acute oral toxicity. If on the basis of these studies a long-term effect could be assumed then additional sub-chronic and chronic toxicity testing will also be required.

In contrast to the situation in the EU GM foods do not require to authorization. Manufacturers, however, usually take advantage of voluntary consultations with FDA – not least because of the strict US liability regime. GM food is only subjected to an authorisation procedure if it is deemed to be neither substantial equivalent nor "generally recognized as safe" (GRAS). Recombinant DNA as well as their respective gene products are generally classified as GRAS, unless they are considered as novel compounds to food or as significantly different from compounds already present in foods.

The US interpretation of the concept of substantial equivalence is however quite different compared to the EU and to the OECD Guidelines of 1996. Products containing recombinant DNA, or their respective gene products are considered to be substantial equivalent. This is also true for products containing altered compounds, e. g. modified composition of fatty acids, if there is preceding experience with these particular fatty acids in foods. A product is not considered substantial equivalent only if the proteins introduced exhibits allergic properties, if an increased level of toxins was detected or if the composition of the food stuff was modified in comparison with foods already on the market. Hence, all GMP commercialised in the USA so far, are considered substantial equivalent.

According to the FDA possible allergenic properties have to be tested if either the protein itself or the donor is known to be allergic. In the US the IFBC decision tree is preferred, which encompasses different procedures in case of allergenic sources compared to the decision trees used in the EU. In contrast to the EU the US scheme distinguishes further between "commonly allergenic" and "less commonly allergenic" donor organisms.

In case of proteins from allergenic donors, both schemes suggest in-vitro tests (RAST, ELISA, immunoblotting) followed by an in-vivo skin-prick test. If the skin-
prick test turns out to be positive, labelling is recommended. If the test leads to negative results, the US scheme proposes a Double-Blind Placebo-Controlled Food Challenge (DBPCFC), whereas the EU scheme suggests in-vitro characterisation on the basis of both homology and stability studies. In case of a negative result both schemes endorse placing on the market, in case of a positive result labelling is recommended.

In case of proteins from less commonly allergenic donors the US scheme enables a shortcut procedure. In case of a negative immune reactivity in the in-vitro immunoassay no further immunological testing (Skin-Prick test, DBPCFC) is required. Still, labelling is recommended.

If the donor is not known to be allergenic, the proposed procedures are similar in both the US and the EU schemes and comprise sequence comparisons to known allergens and digestibility studies.