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Concluding remarks

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Abstract

The most important results from the EU-sponsored ENTRANSFOOD Thematic Network project are reviewed, including the design of a detailed step-wise procedure for the risk assessment of foods derived from genetically modified crops based on the latest scientific developments, evaluation of topical risk assessment issues, and the formulation of proposals for improved risk management and public involvement in the risk analysis process.

Keywords: Food; Plant biotechnology; Genetic modification; Genetic engineering; Genetic manipulation; Transgenic crops; Novel foods; Regulation; Safety assessment; Risk analysis; Toxicology; Allergy, substantial equivalence; Unintended effects; Post market monitoring; Compositional analysis; Advanced analytical methods; Profiling; Horizontal gene transfer; Antibiotic resistance marker genes; Detection; Traceability; Consumer issues; Public participation

1. Objectives of ENTRANSFOOD

The rapid adoption of genetically modified (GM) food crops with improved agronomic characteristics in the US, Argentina, and Canada stands in strong contrast to the situation in the EU. New authorisations in the EU have been suspended since 1999, until a more rigorous and transparent regulatory framework has been put into place, which includes provisions for monitoring, labelling, and traceability. This de-facto moratorium is primarily based on consumer concerns about the safety of GM crops with respect to possible long-term adverse effects on the environment and human health. Doubts have also been expressed on whether the new technology will contribute to a more sustainable agriculture and what impacts technology adoption might have on global agro-food production and society at large. Scepticism of the European consumer towards

Abbreviations: BASIS, BioActive Substances in Food Plants Information System; DNA, deoxyribonucleic acid; ENGL, European Network of GMO Laboratories; EFSA, European Food Safety Authority; ENTRANSFOOD, European network safety assessment of genetically modified food crops; EU, European Union; FAO, Food and Agriculture Organisation of the United Nations; FOSIE, European Concerted Action Food Safety in Europe; GI, gastro-intestinal; GM, genetically modified; GMO, genetically modified organism; GMOBILITY, EU-project on safety evaluation of horizontal gene transfer from genetically modified organisms to the microflora of the food chain and human gut; GMOCARE, EU-project on new methodologies for assessing the potential of unintended effects in genetically modified food crops; GMOCHIPS, EU-project on new technology in food science facing the multiplicity of new released GMO; HGT, horizontal gene transfer; ILSI, International Life Sciences Institute; OECD, Organisation for Economic Co-operation and Development; Qpcrgmofood, EU-project on reliable, standardised, specific, quantitative detection of genetically modified foods; RTD, research and technology development; SAFOTEST, EU-project about new methods for the safety testing of transgenic food; UK, United Kingdom; US, United States; WHO, World Health Organisation of the United Nations.

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GM crops arises also from the relatively low trust in the authority of regulatory agencies in Europe, not least fuelled by recent food crises.

The Thematic Network ENTRANSFOOD started its activities February 2000 in order to respond to some of the concerns raised in regulatory and public deliberations on GM crops. Activities focused on (i) the evaluation of current approaches to risk assessment of GM crop-derived foods and the potential needs for further improvement of safety testing methods for foods, (ii) measures that underpin an effective management of GM crops once entered the market, including legal requirements for detection, labelling, and traceability throughout the food production chain, and (iii) analysis of consumer's attitudes towards GM foods and how concerns can be dealt with during the risk analysis process.

In the ENTRANSFOOD consortium, all the different stakeholders involved in development and production of GM crops and derived foods and in risk-assessment,—management, and—communication were brought together and they committed themselves to debate the above mentioned issues in an open atmosphere and without prejudice. The most important results from this $3\frac{1}{2}$-year project are the design of a detailed step-wise procedure for the risk assessment of foods derived from GM crops based on the latest scientific developments, evaluation of topical risk assessment issues, and the formulation of proposals for improved risk management and public involvement in the risk analysis process.

2. Main achievements

2.1. A step-wise approach to the safety assessment of GM crop derived foods

A detailed stepwise approach has been designed by ENTRANSFOOD to guide the choice of test methods for the safety assessment of foods derived from GM crops (König et al., 2004). The integrated and iterative approach to hazard assessment and safety evaluation of all elements involved in producing a new GM crop variety includes characterisation of (i) the parent crop, donor, transgene(s) and the delivery process, (ii) toxicological characterisation of newly expressed gene product(s), and (iii) a comparison of the new GM crop or derived food to the conventional counterpart. The safety assessment should take into account any changes in food use of the crop that might be affected through introducing a new trait. A critical element in the assessment is the detection of potential unintended or unexpected alterations resulting from the genetic modification; if such unintended effects do occur, their health implications have to be assessed.

The proposed stepwise safety evaluation procedure is an important step forward in risk assessment of the new category of foods, since it adds a significant level of detail to the general requirements for the actual safety assessment. The procedure takes as a starting point a *comparative* approach, relying on the identification of possible differences in agronomical, morphological, and (bio)chemical properties between the unmodified parent line and the GM line: The Concept of Substantial Equivalence (as originally developed by the OECD). Identified differences are subject to further toxicological and nutritional assessment.

Critics have pointed out that application of the Substantial Equivalence principle could be better standardised and harmonised with respect to performance of the field trials and the compositional analysis. Which parameters should be assessed for each crop species, and what are natural variations in key macro- and micro nutrients, anti-nutrients and natural toxins in food crops has been addressed by the OECD Task Force for the Safety of Novel Foods and Feeds (OECD, 2003). In a complementary activity, the US branch of the International Life Science Institute has constructed a Crop Composition Database, which is available on the Internet (http://www.cropcomposition.org; ILSI, 2003). Furthermore, the food plant database BASIS is developed, which assembles the compositional, toxicological, and nutritional data for the 300 most common food plants in Europe (BASIS, 2003). These forces should be united to develop uniform and harmonised criteria for quality assessment of the data and the analytical methods.

Several in silico-, in vitro-, and in vivo-test methods developed for safety testing of single chemicals, food additives, and contaminants, recently reviewed by FOSIE (Renwick et al., 2003), can be used, sometimes after adaptation, to characterise recombinant proteins and metabolites in foods derived from GM crops. Toxicity studies with *whole* foods in laboratory animals can yield meaningful information and add to the safety assurance of the new food, notwithstanding certain limitations in establishing dose-response relationships and the possible occurrence of confounding effects. The need for toxicity tests is to be determined on a case-by-case basis.

Strategies for assessing the potential allergenicity of GM crops, recently adopted by the Codex Alimentarius (Codex Alimentarius Commission, 2003), concentrate on characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce de novo sensitisation or to elicit allergic reactions in already sensitised persons, and whether the transformation may have altered the allergenic properties of the modified food. A *weight of evidence* approach is recommended, taking all information obtained with various test methods into account, since no single experimental method yields decisive evidence for allergenicity.

A further refinement of testing of whole novel foods is examined in the SAFOTEST project (Knudsen, personal

communication). A combination of in vivo animal models, in vitro toxicological systems, and selected profiling methods is used to characterise GM rice containing *Phaseolus* (PHA-E) lectin. Repeated dose studies in rats are performed with diets containing the parental rice, the GM rice, or the GM rice spiked with the purified lectin (in lowest-observed-adverse-effect-level dosage) after an initial study where the dose–response toxicity of the lectin is established. These experiments are paralleled by in vitro experiments on the digestibility and cytotoxicity of the recombinant proteins, using intestinal epithelial cell lines derived from humans and rats. Gene expression profiles in rat and human intestinal epithelial cell lines and in parallel in intestinal samples from live rats are determined upon exposure to lectins using DNA microarrays. This approach provides interesting information on the potential correlation of results obtained in the different test systems of animal and human origin and may provide a guide to future approaches to establish the safety of GM food as such.

The detailed stepwise safety assessment procedure for GM crop derived foods as proposed by ENTRANSFOOD provides a high level of safety assurance for these new types of foods. Foods that have gone through this testing paradigm without adverse findings can be considered as being at least as safe as their conventionally produced counterparts. During the ENTRANSFOOD meetings, it was argued that foods from GM crops evaluated in this way are better characterised and safeguarded for the consumer than conventionally-developed new crops.

The ENTRANSFOOD strategy is also suitable for future GM crops with more complex changes in the metabolism and/or developmental pathways, such as nutritionally enhanced crops, and crops with improved performance under environmental stress conditions (salt tolerance, cold/heat resistance), in cases where a suitable comparator is available. In case such crops cannot be compared with conventionally grown ones due to extensive alterations in composition or agronomical behaviour, a more comprehensive case-by-case assessment of their safety has to be carried out.

The issue of tracing potential adverse effects in humans due to the consumption of GM foods has also been addressed by ENTRANSFOOD. Post-market monitoring of adverse effects from intake of specific foods seems feasible if specific adverse effects (e.g. allergenicity) and distinctive symptoms to be looked for can be identified. Still the success of a post-market monitoring of foods is strongly dependent on whether exposure to the food item can be estimated with reasonable accuracy and whether any reported effects can be matched with consumption of the food product (Wal et al., 2003). This may be feasible for identity preserved products, but difficult—if not impossible—for commodities.

A recent study commissioned by the UK Food Standards Agency has examined the feasibility of using supermarket—and household survey—data for post-market monitoring of novel foods (FSA, 2003). Food purchase data of British households between 1991 and 2000 were analysed regarding different marker products and a significant variation in purchase behaviour by socio-economic subgroup and region was noted, suggesting that it may be feasible to detect variations in exposure to specific food products, which subsequently may be related to health outcomes.

In the view of ENTRANSFOOD, post market monitoring of adverse effects from intake of food should only be decided for on a case-by-case basis and should be based on specific hypotheses.

2.2. Detection and assessment of unintended effects

The possible occurrence of unintended alterations in the composition of GM food crops as result of the genetic modification is one of the key issues evaluated by ENTRANSFOOD (Cellini et al., 2004). Unintended effects on the concentrations of plant constituents may also occur in classical plant breeding through recombination or mutagenesis events. Such alterations have not created serious food safety problems, since early detection led to specific variety and progeny selection and resulted in substantial decrease or even complete removal of such compounds.

Detection of unexpected effects in GM food crops relies primarily on a *targeted* approach, i.e. comparative determination in GM and non-GM products of levels of *selected* macro- and micro-nutrients, anti-nutrients, and known toxins, which are part of various intrinsic biochemical pathways in the plant and thus may serve as markers for potential alterations. The availability of nutritional and toxicological information of known components drives in many cases the choice of compounds to be targeted. Limitations of this approach lie in the still fragmented knowledge of plant metabolic pathways and lack of knowledge of consequences of the transformation process on possible compositional alterations. The possibly restricted and 'biased' selection of compounds for the detection of unexpected alterations is recognised and therefore a broad selection of compounds representative for diverse metabolic pathways in the plant is advocated.

In order to increase the probability of detecting unintended effects, '*profiling*'- or '*omics*'-techniques have been further developed within the GMOCARE project (Noteborn et al., personal communication). These evolving technologies include transcriptomics, proteomics, and metabolomics and enable measurement of thousands of compounds in modified and unmodified plants, which are not defined prior to analysis (*non-targeted approach*). Research has concentrated on various

transgenic potato lines modified in their starch, amino acid, or glycoalkaloid metabolism and on transgenic tomato lines with elevated phytosterol or isoprenoid contents. These methods are promising, but need much further development and validation before they can be used in a formalised risk assessment procedure. One of the key challenges in using the 'omics' techniques is the development of data processing tools and the establishment of interconnected databases containing profiles that reflect natural compositional differences between genotypes of the same species and different environmental conditions.

Comparative *targeted* analysis of GM and non-GM crops offers a high degree of certainty with respect to detection of unexpected compositional alterations, and is the leading approach in risk assessment of GM crop derived foods. Profiling methods should not be seen as substituting classical targeted approaches, but rather as tools that may increase our understanding of metabolic pathways and their interconnectivities. Further reduction of uncertainties of unintended effects from genetic modification will come from the rapid expansion of knowledge of plant genomes and functions of individual genes, and access to databases with DNA sequence information.

2.3. Risk assessment of gene transfer

Horizontal gene transfer (HGT) of recombinant DNA from GM crop-derived foods to humans and its consequences for human health is an important issue when evaluating the safety of the new foods (Van den Eede et al., 2004). A crucial question is whether the newly inserted genes in the food crop may be taken up by the human gut microflora or the human or animal genome and what would be the consequences. It should be realised that gene transfer amongst different organisms is quite common in nature and has been a driving force in evolution. The transfer of recombinant DNA from foods derived from GM crops to microbes or human cells upon ingestion is a rare event and only consequential if the trait is expressed and confers selective advantage.

Concern has been raised about the use of antibiotic resistance genes in GM plants regarding a potential transfer of these genes from the plant material to micro-organisms which may lead to an increased level of resistance towards antibiotics in micro-organisms. This may pose a risk to human or animal health by compromising the therapeutic value of antibiotics for treatment of pathogenic micro-organisms.

The discussion on the use of antibiotic resistance marker genes in transgenic plants is topical in the European Union since the new Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms (GMOs) states that GMOs that contain genes expressing resistance to antibiotics in use for medical or veterinary treatment should be taken into particular consideration when carrying out an environmental risk assessment with a view to identifying and phasing out antibiotic resistance markers that may have adverse effects on human health and the environment.

ENTRANSFOOD has evaluated this problem and proposes a classification of antibiotic resistance genes based on their distribution in soil and enteric bacteria and the present state of therapeutic importance of the relevant antibiotics. Three categories of marker genes can be distinguished: (I) antibiotic resistance genes that are already widely distributed among soil and enteric bacteria and that could confer resistance to antibiotics that have no or only limited therapeutic relevance in human and veterinary medicine; (II) antibiotic resistance genes that are widely distributed in micro-organisms and that could confer resistance to antibiotics that are used for therapy in certain defined areas of human and veterinary medicine; and (III) antibiotic resistance genes that could confer resistance to antibiotics relevant for human or animal therapy.

The presence of category I genes, like neomycin phosphotransferase (*npt II*) and hygromycin phosphotransferase (*hpt*), would not contribute to the spread of resistance in the environment and therefore not pose an additional environmental or human health risk. Category II genes may add little to the spreading of antibiotic resistance, but these antibiotics are still used in human medicine and might become even more important in the future in case of increased resistance against alternative antibiotics. Category III genes may interfere with the therapeutic function of important (reserve) human antibiotics and their presence in transgenic food crops should be avoided. Thus Category I markers may continue to be used for commercial applications of GM crops in the EU. Phasing out of these markers would represent a significant set back in research and development of GM plants.

The GMOBILITY project has studied the effects of food—and gastro-intestinal (GI) tract—environments on the integrity of DNA using various animal models and a computer-controlled gastro-intestinal simulation model (Van der Vossen et al., personal communication). The digestion process in the GI tract affects rapidly the availability of DNA for transformation. HGT could be demonstrated via transformation when applying a marker rescue system under optimal conditions, but could not be shown in a natural situation. Thus transformation of bacteria in the food chain cannot be excluded when homologous sequences are applied, which may recombine upon entrance of the bacterium. This stresses the point that the presence of bacterial homologous sequences in transformation constructs for food crops should be kept to a minimum.

Whether 'foreign' DNA can be taken up by mammalian cells has also been examined by ENTRANSFOOD, since fragments of bacteriophage DNA fed to mice were found in various cell types, while evidence has also been presented for transplacental transfer in pregnant mice. It should be emphasised that: (i) humans and animals have consumed DNA of various origins throughout history with no evidence for the presence of DNA sequences of plant origin in mammalian genomes, and (ii) DNA uptake is much more probable for somatic cells, particularly those of the gut—and immune—systems, than for germ line cells. Since the somatic cells of the gut lining have a rapid turnover, chances of adverse consequences are highly unlikely. Although uptake of GM crop-derived DNA by human cells cannot completely be ruled out, it is very unlikely that recombinant DNA is taken up and expressed in germ line cells.

2.4. Further research needs to improve the risk assessment of GM foods

2.4.1. Improved gene delivery methods

Methods for crop transformation should be improved in order to reduce the amount of recombinant DNA introduced into the GM crop. This can simplify and reduce uncertainties in the safety assessment of GM crops (see König et al., 2004, König, 2003).

2.4.2. Improved safety and nutritional test models for foods

Advances in molecular biology will continue to progressively increase our understanding of the metabolic and cellular processes that determine dietary health and safety relationships. Based on this knowledge new toxico-genomic and nutri-genomic models can be developed, which may provide sensitive biomarkers for biological responses of humans and animals upon consumption of food components and whole foods. These new methods may provide specific information on shape of dose-response curves and on the existence of threshold levels for toxic and beneficial effects. Moreover, interspecies differences in effect responses may be elucidated at the molecular level.

2.4.2.1. Allergenicity. The development of robust methods for identifying and characterising the potential of proteins to cause IgE-mediated allergic sensitisation would be facilitated by a more detailed appreciation of the features that confer allergenic activity on proteins. This is of great importance, since future GM crops may contain proteins derived from sources to which humans never have been exposed.

There is a need to understand in greater detail the ways in which variables like protein folding, glycosylation, or phosphorylation may affect the ability to induce allergic sensitisation and what degree of sequence similarity with known allergens signals a likely hazard.

It is important to define the structural motifs of T- and B-lymphocyte epitopes on proteins, which are associated with the initiation of allergic responses, and still little is known about thresholds of both sensitisation and elicitation.

Although a correlation between resistance to proteolytic digestion and allergenic potential has been proposed, the association is not absolute and therefore the extent to which there exists a relationship between allergenic potential and resistance to proteolytic digestion should be further explored.

Several animal models have been proposed and some of these show promise either as methods for the assessment of the allergenic potential or for more mechanistic studies, but none of these models have yet been formally evaluated or validated. Research should be continued into the immuno-biology of protein allergy with particular emphasis on the identification of molecular markers that can be used to distinguish protein allergens from non-sensitising proteins. Microarray and proteomic technologies may be appropriate for this purpose and also for determining whether transformation has caused any unintended changes in the level of expression of allergenic proteins endogenous to the host plant.

2.5. Detection and traceability

Legal requirements for provisions to trace back food ingredients derived from GM crops through the agro-food chain have two main objectives: enabling withdrawal of foods or feed derived from GM crops in case of the appearance of unforeseen adverse effects, and providing consumers choice between foods containing—or free of—GM crops. To this end, labelling of foods derived from GM crops containing detectable transgenic material is required by EU legislation since 1998 (EU Regulations 1139/98 and 49/2000). Recently adopted legislation (EU Regulations 1829/2003 and 1830/2003) will extend this labelling to foods without any traces of transgenics. This new legislation will also impose labelling and a traceability system based on documentation throughout the food and feed manufacture system. The implementation and maintenance of the regulations necessitate sampling protocols and analytical methodologies that allow for accurate determination of the content of GMOs within a food and feed sample. These issues are discussed in the paper by Miraglia et al. (2004).

Two RTD projects concentrate on the development of detection methods for GM crop-derived foods.

The Qpcrgmofood project (Holst-Jensen, personal communication) has focused on the development of quantitative methods for GM food crops, as well as on new strategies for method development and validation,

in particular of multiplex methods and specific real-time PCR methods, and identification of specific reference genes for the most important GM food crops. The GMOCHIPS project (Remacle, personal communication) has focused on the development of detection methods using the microarray technology. Biochips have been constructed containing specific and control genetic elements present in GM food crops. Quantification, high throughput upscaling, and validation are in progress.

Crucial for the development of any GMO detection and/or identification method is the availability of sequence information on the genetic modification, as well as relevant reference materials. This requires global exchange of information of GM events on the market and in development. An important step within Europe in this respect has been the establishment of the European Network of GMO Laboratories (ENGL). The increasing number of GM varieties being tested in the field and/or released within the EU and abroad demands robust methods to detect *multiple* GMO events at once.

It is considered unavoidable that non-GMO—and authorised GMO—derived raw materials may become mixed, especially during transport of bulky products. This mingling is practically very difficult, if not impossible, to avoid and this forms the rationale behind the establishment of the current 0.9% threshold level for the labelling of GM varieties in a GMO-free bulk. There are, however, technical constraints and uncertainties related to the maintenance of this threshold level, such as units to be compared (DNA/DNA or weight/weight?), availability of reference materials and GMO event-specific sequence information, and presence of "stacked genes" and unauthorised GMOs.

The proposed regulatory EU requirements for traceability of GM products fit within a broader tendency towards traceability of foods in general. Traceability systems document the history of a product and specific segregation and identity preservation systems allow for the separation of GM and non-modified products from "farm to fork". The feasibility of traceability systems depends on a number of factors, including unique identifiers for each GM product, availability of detection methods, permissible levels of contamination, and financial costs. For success, much will depend on the threshold level for adventitious contamination set by legislation. Such systems may reduce the necessity for stringent sampling schemes, but the administrative burden of the documentation should not be underestimated.

2.6. Consumer attitudes towards GM food crops

Societal responses to the application of technological innovations may, besides concerns about possible effects on the environment or human health, be driven by concerns about the impact of the technology on society. In order to understand how people's attitudes and values influence their acceptance or rejection of GM foods, their attitudes towards science and technology per se should have been analysed (Frewer et al., 2004). Simply providing more information on the risks and benefits of GM crops is unlikely to effect changes in consumers' attitudes.

A number of surveys (Eurobarometers) have been conducted in the last decade to gauge the overall level of consumer attitudes towards biotechnology in the EU. Results of the survey held in 2000 indicate that Europeans seem to have relatively neutral attitudes towards GM foods as a technology. Interestingly, these attitudes tend to be more positive where the "first generation" of GM foods is concerned and more negative where the "second generation" is concerned. Moreover, there appears to be a downward trend over time. When compared to the results of the previous survey held in 1996, consumers' attitudes to both generations of GM foods have become more negative in terms of usefulness and moral acceptability, but have remained constant regarding risk perception.

Public concerns ranged from fears like allergies, outcrossing, and development of super-weeds, to worries prompted by uncertainty, i.e. unintended effects on human health and the environment, and the potential irreversibility of any negative impact. Besides concerns with the outcomes of technical risk assessments, also concerns about the *uncertainty* related to risk assessments have been expressed. Furthermore, research has elucidated the details of moral concerns, highlighting issues like unnaturalness, "tampering with nature", animal welfare, the power balance between producers and consumers, democracy, and disparity between the industrialised world and the Third World.

If public confidence is to be regained, it is important to explicitly incorporate public concerns into the risk analysis process. Once public concerns and the values on which they are based are understood, they can be more effectively introduced into risk assessment and risk management practices. Different tools such as technology foresight, citizen panels, focus groups, and future search conferences are available in order to make an active involvement of the public possible.

3. Implications of ENTRANSFOOD results for risk analysis of foods derived from GM crops

1. ENTRANSFOOD was primarily designed to deal with food safety and consumer concerns related to the introduction of foods derived from GM crops. However discussions during the meetings pointed out that consumer concerns regarding a possible negative impact of large-

scale cultivation of GM crops on the environment have equal weight. This was not covered by ENTRANSFOOD, but a global risk analysis of the technological innovation in agriculture should include these aspects.

2. The ENTRANSFOOD model has been a unique experience with participants with different disciplines and from different affiliations, which made it possible to jointly examine a broad range of relevant issues related to the risk assessment, management, and communication of GM crop-derived foods. In this way, consensus was reached that a rigorous science-based risk assessment of the environmental impact and of possible effects on human health of foods derived from GM crops only is not sufficient to gain public support for the introduction of this new food production technology into the society. Appropriate risk management measures and aspects like sustainability, benefits, and impact on the society must be taken into account. The need for further development of new approaches for risk analysis that integrate these aspects, as signalled by ENTRANSFOOD, has already been honoured in the 6th Framework Research Programme in the call for food safety projects.
3. ENTRANSFOOD has added a significant level of detail to the current guidelines for the scientific pre-market assessment strategies for GM crop-derived foods. The designed stepwise procedure could serve as a reference standard for data generation and risk assessment in the framework of the new EU Regulation on GM food and feed (1829/2003). The refined assessment approach could also serve as a basis for refinement of international guidelines for the food safety assessment of GM crops developed by intergovernmental organizations like OECD and FAO/WHO. The adoption of a more detailed standard procedure would contribute to harmonize risk assessment and management in the public and private sector alike. Post-market monitoring is only seen as an instrument for targeted consumer studies on specific grounds, such as qualified suspect for allergenicity.
4. ENTRANSFOOD has examined existing and evolving methods to detect the possible occurrence of unintended effects on the composition of GM food crops as result of the genetic modification. Detection of such effects should primarily rely on *targeted* approaches with measuring single compounds in GM and non-GM plants that represent important metabolic pathways. This should continue to be the leading principle in regulatory risk assessment procedures for GM crops and derived foods and feed. Profiling techniques, although potentially powerful tools to screen for unintended effects, are not considered alternatives for targeted analysis, given the infant state of development and validation. It is far too early to apply these techniques in formalised risk assessment procedures.
5. ENTRANSFOOD has examined the issue of whether genes coding for antibiotic resistance can be used as markers for the transformation event. The public debate is focused on hazards of transfer of these genes present in GM crop-derived foods to microbes residing in the human gut or to human cells. Although the actual risk of transfer and subsequent spreading of resistance is deemed negligible, a precautionary approach with the use of these marker genes is advocated, since there is still a lack of knowledge regarding specific conditions where gene transfer might be possible. ENTRANSFOOD has categorized currently available marker genes into three groups, according to their presence in the environment and the importance of the antibiotic for clinical use. This implies that the presence of certain marker genes like *nptII* and *hpt* in GM crops do not pose an additional risk to the environment or human health, and thus the use of these genes as markers can be continued. This risk classification is aimed at contributing to the current debate within the European Commission regarding the phasing out of those marker genes that pose a threat to the environment or human health (EU Directive 2001/18).
6. ENTRANSFOOD has identified a number of issues that need further research and these topics may be considered by national and international organisations for funding. Priority issues are:
   - Improvement of transformation methods and of methods for marker gene elimination
   - Development of improved test protocols for animal feeding trials with whole foods
   - Development of toxico- and nutri-genomic models for safety and nutritional assessment of new foods using gene expression and other profiling technologies
   - Further development and validation of profiling methods and the set-up of interconnected databases containing profiles of important food crops
   - Research on mechanisms of allergenicity, protein stability, sequence homology, and development of animal models,
   - Feasibility studies of post-market monitoring of foods derived from GM crops
   - Development of new strategies for detection of GM material in food crops and derived foods by using high-throughput multi-analyte

detection methods (microarray, mass spectrometry, surface plasmon resonance)
- Feasibility studies of effective and trustworthy traceability systems for GM crops and derived foods
- Research on new ways of public participation in the risk analysis process for foods and new food producing technologies

7. The establishment of a *Permanent Evaluation and Discussion Platform* as a follow-up of ENTRANSFOOD for the scientific and societal assessment of the development and introduction of future foods in Europe should be considered. This Platform or Virtual Centre could focus on risk/benefit analysis of new foods produced by different methods and under different agricultural practices. Key players in risk analysis and communication from industry, academia and consumer organisations may consider participating in this initiative. One of the goals would be to design a general framework for risk/benefit analysis of foods and food production systems taking the new scientific developments and societal aspects into account. Furthermore, the Platform could advise the European Food Safety Authority (EFSA), and provide guidance for the development of the food safety and nutrition research agenda in Europe.

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