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Genetically modified crops in the EU: food safety assessment, regulation, and public concerns

Overarching report
Entransfood, the European network on safety assessment of genetically modified food crops

www.entransfood.com

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PREFACE

Approaches to the food safety assessment of foods derived from genetically modified (GM) crops have been developed over the last two decades by intergovernmental organisations, including the Organisation for Economic Cooperation and Development, as well as the United Nations' World Health Organisation (WHO) and Food and Agriculture Organisation (FAO). The European Commission has made substantial contributions to research and expert deliberations in this area: over the last twenty years, it has supported 81 projects on the assessment of environmental and food safety of genetically modified organisms (GMOs) and derived foods; contributing approximately 70 million Euro (see Review of Results of EC-sponsored Research on Safety of Genetically Modified Organisms, 2001, http://europa.eu.int/comm/research/quality-of-life/gmo).

Despite these intensive research efforts assessing the safety of GM crops, European consumers remain apprehensive. Consumer and environmental organisations have voiced concerns about the safety of these crops with respect to long-term effects on the environment and human health, as well as consumers' freedom of choice between GM containing and 'GM-free' foods. The media coverage of this debate demonstrates that a rigorous science-based risk assessment may not suffice to introduce a new food production technology into society, but that *societal* aspects should also be taken into account. The public debate on GMOs is part of a more general discussion on the safety of foods produced in Europe, fuelled by the BSE and dioxin crises, which have resulted in low public trust in food safety assessment and management practices in Europe.

The overarching objective of the Thematic Network ENTRANSFOOD was to address scientific and societal issues related to the adoption of GM food crops. The Network consisted of five research projects and five Working Groups, which focussed on the development of methods and strategies for safety testing, detection, and traceability of GM food crops, as well as societal aspects of the introduction of GM foods and started its activities in February 2000 (**www.entransfood.com)**.

ENTRANSFOOD provided a platform for participants from a wide range of different perspectives and disciplines to interact and to explore the interdependence of scientific, regulatory, and societal aspects of introducing GM food crops. Project participants and sponsors consider ENTRANSFOOD a trial model to inform future deliberations on how to structure multidisciplinary research projects on questions relating to risk. Integration of scientific, regulatory, and societal aspects allowed addressing food safety in an interdisciplinary manner; in the wake of the BSE crisis this is now considered essential for the introduction of new food producing technologies into the society. Several members of ENTRANSFOOD have contributed over the last years to the activities of expert panels, expert consultations, workshops, and congresses addressing the safety of foods derived from GM crops. Participants have also participated in public meetings and hearings to communicate on the safety of foods derived from GM crops and other aspects of agricultural biotechnology. These activities intend to place the consumer in a better position to judge the potential risks of GM foods and the impact of the new technology in the society.

Objectives of the Thematic Network were:

- To identify key issues of the safety evaluation of foods derived from GM food crops, and to examine whether current research methods are adequate to characterise specific safety hazards;
- To evaluate current food safety assessment strategies, and to identify differences in approaches and interpretation;

- To design and evaluate new alternative (in-vitro) test methods for the safety evaluation of GM food crops;
- To provide detailed guidance to notifiers and risk assessors to perform the safety assessment of GM food crops;
- To assess the risks of transfer of recombinant DNA from GM crops to microbes or human cells;
- To examine the fate of GM raw materials and processed products throughout food production chains (traceability);
- To examine new strategies for the detection of GM raw materials, processed products, and food ingredients;
- To examine societal aspects and consumers attitudes towards the introduction of foods derived from GM food crops;
- To establish a communication platform of producers of GM foods, scientists involved in food safety research and in societal aspects of GM food introduction, regulatory authorities, retailers, and consumer groups.

Participants of the ENTRANSFOOD Consortium were recruited from academia, research centres, biotech and breeding companies, food industries, food retailers, regulatory agencies, and consumer groups across Europe. Forty-five Research Centres participated in the RTD projects, and 62 experts in the Working Groups. Many of the Working Group members are also actively involved in the research projects. Total costs involved in ENTRANSFOOD are € 12.302.449, with an EU contribution of € 8.390.776.

Structure and Working Procedure of ENTRANSFOOD (see Figure Ex-1)

Research was carried out in five European Commission-funded shared cost projects involving researchers from the public and private sector:

1. New methods for the safety testing of transgenic food (SAFOTEST, QLRT-1999-00651).
2. New methodologies for assessing the potential of unintended effects in genetically modified food crops (GMOCARE, QLK1-1999-00765).
3. Safety evaluation of horizontal gene transfer from genetically modified organisms to the microflora of the food chain and human gut (GMOBILITY, QLK1-CT-1999-00527).
4. Reliable, standardised, specific, quantitative detection of genetically modified foods (Qpcrgmofood, QLK1-1999-01301).
5. New Technology in Food Science Facing the Multiplicity of New Released GMO (GMOChips, G6RD-CT2000-00419).

Evaluation and review activities have been carried out in five Working Groups:

1. Design of safety assessment strategies for transgenic foods
2. Design of strategies for the detection of unintended alterations in GM food crops due to the process of genetic modification
3. Evaluation of the risks of gene transfer from GM foods to micro-organisms in the human digestive tract or to human cells
4. Evaluation and design of strategies for detection and traceability of GM foods and food components
5. Understanding of societal responses to GM foods

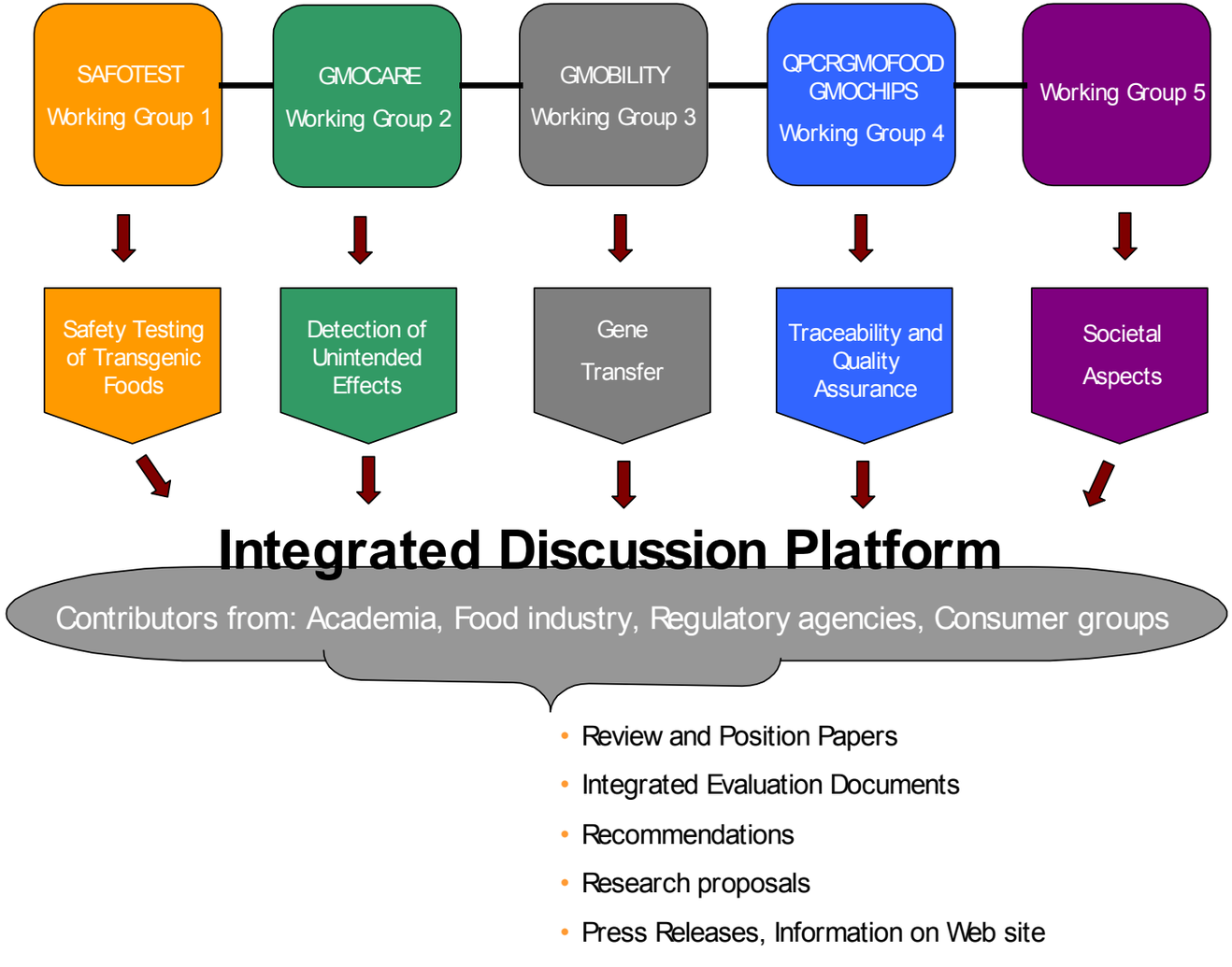

Figure Ex-1: Structure of ENTRANSFOOD activities

The project was initiated in February 2000 with an inaugural meeting in Wageningen, the Netherlands, and concluded with a Conference in May 2003 in Rome. Plenary meetings of ENTRANSFOOD were held twice a year in order to (i) outline and further develop the project strategies, (ii) report on progress of the research projects, (iii) report on progress of the Working Groups, and (iv) establish inter-project and Working Group exchanges. Working Group meetings were held in connection with the plenary meetings or separately. Each research project was independently co-ordinated and held separate progress meetings. Two Integrated Discussion Platform Meetings were held with invited experts from various stakeholder groups to comment on the progress of ENTRANSFOOD; their inputs shaped the subsequent course and outcomes of the Network.

Results of the RTD projects have been and will be published in peer reviewed scientific journals. Each Working Group will publish a scientific paper in a special issue of the journal Food and Chemical Toxicology. This European Commission–published Overarching Report summarises the main findings of the Working Groups to inform stakeholders, policy makers, consumer groups, and interested public. All ENTRANSFOOD participants have contributed to writing the paper.

Extension of the project by six months allowed increased focus on integration of societal aspects and scientific and regulatory aspects. The main lesson from this project is that the interaction of all members of diverse working groups with diverse backgrounds needs to be attended to in a proactive manner, possibly by considering establishment of more formal organisational structures and processes for deliberation on cross-cutting issues, such as the definition and assessment of uncertainties about specific aspects of the safety assessment and the implementation of regulations.

Future activities on risk analysis of new food production technologies and food production systems need similar integrated approaches and can further build on ENTRANSFOOD experiences. In fact, the establishment of a Permanent Evaluation and Discussion Platform on GMOs in Europe as a follow-up of ENTRANSFOOD would significantly contribute to further development of risk analysis models, which must include new effective procedures for public involvement in the risk analysis process.

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EXECUTIVE SUMMARY

1. ENTRANSFOOD has identified and discussed the main issues concerning the introduction of foods derived from GM crops. These issues are (i) safety testing methods; (ii) unintended effects; (iii) gene transfer; (iv) detection, labelling, and traceability; (v) consumer acceptance; (vi) regulatory framework; and (vii) interdisciplinary deliberations.

2. Worldwide, the consumption of foods derived from genetically modified crops (GM crops) is rising rapidly. Since the first GM crop was introduced in the market for large-scale cultivation in 1996, the global area of cultivation has risen to 67.7 million hectares in 2003 (James, C., 2003. Global Status of Commercialized Transgenic Crops: 2003, ISAAA Briefs No. 30: Preview, International Service for the Acquisition of Agri-biotech Applications, Ithaca). In 2003, GM soybeans, maize, cotton, and oilseed rape accounted for 61%, 23%, 11%, and 5% of the total global acreage of GM crops, respectively. The first generation of GM crops contain new genes that protect the crop against certain insect pests, or that confer tolerance to broad-spectrum herbicides facilitating weed control. The rate of adoption varied across countries: plantings in the US, Argentina, and Canada accounted for 63%, 21%, and 6% of the total global area of cultivation. In Europe, GM crops were grown in Romania (70,000 hectares of GM soybean), Spain (32,000 hectares of insect-protected maize), Germany, and Bulgaria. Genetic modifications of crops that are being developed for future commercialisations include more complex modifications, such as enhanced stress-tolerance or nutritional characteristics.

3. The much slower adoption of GM crops in Europe demonstrates that rigorous safety assessment is necessary but not sufficient for gaining societal acceptance of agricultural applications of biotechnology. If public confidence in science, technology, and food safety is to be regained, it is important to take consumer concerns and attitudes into account in risk analysis. The agro-food chain consists of stakeholders with diverse interests, including farmers, traders, distributors, processors, retailers, and end-consumers, while the interests of each stakeholder may differ from one market to another. Improved processes for stakeholder engagement in deliberations on technological risks are required.

4. ENTRANSFOOD has brought together representatives from academia, research centres, biotechnology and breeding companies, food industries, food retailers, regulatory agencies, and consumer groups across Europe to address food safety and societal issues of the introduction of foods derived from GM crops.

5. Safety considerations for foods derived from GM crops are fundamentally the same as those for conventional foods. Scientists concerned with product development and regulation in general rely on the same approach for the safety assessment of foods derived from GM crops. The approach defines the type of data to be considered in the safety assessment of individual products. Each individual GM crop is compared to an appropriate comparator that is generally accepted as safe for food use, based on its extensive prior use as a human food.

6. ENTRANSFOOD's deliberations are grouped under five headings: the safety assessment of foods derived from GM crops; identification of unintended effects from genetic modification; assessment of risks emanating from gene transfer across species; development of detection methods, standards for labelling and traceability, and their implementation; and societal aspects of adopting GM crops in the agro-food chain.

The safety assessment of foods derived from GM crops

7. Working Group 1 developed a systematic approach how to tailor the safety assessment of foods derived from GM crops to the specific characteristics of the modified crop and the introduced trait. The corner stone of the safety assessment of foods derived from GM crops is the concept of substantial equivalence. This concept prescribes the comparison of the GM crop to a suitable comparator with a long history of use that allows the identification of any significant differences that might impact human health. These differences then become the focus of further analytical, toxicological, or nutritional analyses. The safety assessment of foods derived from GM crops is divided into four steps: characterisation of the parent crop; characterisation of the transformation process; toxicological evaluation of new gene product(s) and allergenicity assessment; and nutritional, toxicological, and allergenicity evaluation of the GM crop/derived food. The objective of the tests is to assess whether foods derived from GM crops are as safe as foods produced from conventional crops.

8. ENTRANSFOOD recommends the concept of substantial equivalence, which has been widely adopted in both the public and private sector, as the best available approach to safety assessment of GM crops. Contrary to what the critics say, guidelines to its implementation are becoming evermore standardised and detailed. For example, the Organisation for Economic Cooperation and Development (OECD) is compiling consensus documents for certain crop species that provide the information considered of most relevance for the characterisation of the parent crop. These consensus documents guide the application of the concept of substantial equivalence.

9. Discrete substances, such as newly introduced recombinant proteins and metabolites, are characterised by describing what is known about their structure and function. Classic toxicological methods were originally developed for the safety assessment of chemicals in foods, such as food additives. These toxicological methods, including toxicity studies in animals, can be applied for the assessment of the characterised substances.

10. ENTRANSFOOD recommends to conduct repeated dose studies with recombinant proteins or derived substances to identify potential adverse long-term effects unless there is sufficient information to confirm the lack of toxicity or pharmacological activity of the recombinant proteins and metabolites, or if there is extensive experience with these substances (for instance from a history of safe use).

11. The current approach recommended by experts under FAO/WHO and the Codex Alimentarius for the assessment of the potential allergenicity of GM foods involves five steps: the characterisation of the source organism of the novel protein; the analysis of amino acid sequence similarity of the protein and known allergens; study of physico-chemical properties; where the protein is derived from an allergenic source organism or where there are other indications of potential allergenicity, such as structural similarity to known allergens, these tests are complemented with immunological tests and, where deemed appropriate, further investigations. Methods for the assessment of the sensitisation potential of proteins need to be improved, such as through the development and validation of animal models, to allow the transfer of proteins that might share certain structural or physico-chemical characteristics with allergens. The possibility of changes in the allergenicity of the whole crop should also be considered.

12. A more detailed understanding of protein allergy will enhance further safety assessment of a protein's potential for allergic sensitisation. ENTRANSFOOD considers of particular value

the development of an animal model that would permit the identification and characterisation of potential food allergens. Progress in this area will be facilitated by a more thorough appreciation of the factors that confer to proteins the potential to induce allergy and what distinguishes these from non-allergic proteins. Further research on the structure-function relationship of allergens is encouraged.

13. The comparison of the GM crop to its counterpart relies on a targeted approach of parameters indicative of the overall plant metabolism and possible changes from genetic modification that might have health implications. This involves the assessment of composition, physiology, morphology, and agronomic performance. Such targeted approaches can identify unintended changes from the genetic modification. Parameters for comparison are usually indicators of the functioning of major physiological and biochemical pathways of the crop. The focus on key nutrients and anti-nutrients in food crops corresponds to the focus on identifying those changes in food crops that may have implications for human health.

14. Animal tests with whole foods derived from GM crops are considered to contribute with useful information to the safety assessment. We recommend such tests should only be a requirement in cases where the composition of the GM food crop differs significantly from that of its unmodified counterpart or if other tests provide any indications of a potential hazard associated with the genetic modification. In these cases, dietary sub-chronic rat studies (usually, these are of 90 days duration, assessing the classic toxicological endpoints) are recommended to demonstrate the safety of the food. If adverse effects are observed, further toxicological studies on long-term effects should be considered in cases where the product is still deemed fit for marketing. Further standardisation of test protocols for animal feeding trials with novel foods, including foods derived from GM crops is recommended, including recommendations on when, how, and how often the diet is administered, performance of the animal experiment, and choice of toxicological and nutritional endpoints.

15. The described approach to safety assessment is also applicable to new generations of GM food crops with extensive compositional changes. For GM crops that have been modified extensively such that there is no single crop that is a conventional counterpart suitable for comparison, all new substances or existing substances whose levels have been altered should be assessed on a case-by-case basis; safety studies with the whole crop should also be conducted. The safety assessment of GM crops that are intentionally designed to be compositionally different requires increased attention to two issues: the choice of an appropriate comparator and the estimate of the anticipated exposure. One example of a compositionally altered GM crop currently under regulatory review is oilseed rape that contains lauric acid, a fatty acid not normally found at elevated levels in oilseed rape oil. The product was developed as a substitute for tropical oils (for instance, palm oil) in certain food applications. The comparator with safe use in this case was palm oil.

16. ENTRANSFOOD considers the current safety assessment approach adequate to determine whether foods derived from GM crops are as safe as their conventional counterparts. The issue of long-term effects of the consumption of foods derived from GM crops has been addressed by the FAO/WHO Expert Consultation held in 2000, and was endorsed by ENTRANSFOOD. Very little is known about the potential long-term effects of *any* food, and such effects are difficult to assess at the population level due to the complex and changeable diets that prevent attributing specific health effects to individual food components, as well as the variable susceptibility to diverse health impacts across individuals within a population. On a case-by-case basis, randomised controlled trials in humans could be performed to investigate medium/long term effects of foods.

17. ENTRANSFOOD does not recommend post-market monitoring of foods derived from GM crops as a routine practice. Such studies with commodity crops are unlikely to provide meaningful information. Post-market monitoring might be considered for identity-preserved GM crops with changed nutritional characteristics in order to confirm the pre-market safety and nutritional assessment. A clear test hypothesis in form of a causal relationship of food intake and health impact must be formulated.

18. Genomic research adds a new dimension to our understanding of plant biology and provides powerful new tools to study induced changes in gene expression. Increased knowledge of the structure of plant genomes, functions of individual genes, and a plant's responses to its environment at the molecular level will improve our understanding of the characteristics of the parent crop that pertain to food safety assessment of GM crops. The establishment of international systems for improved access to crop genome databases and latest bio-informatics methods in order to facilitate and harmonise the future analysis of such data are key.

19. The availability of sequence information of entire plant genomes also allows for the development of micro-array systems to assay induced changes in gene expression patterns. This will in future also allow assessing potential changes in gene expression in genomic regions in proximity of the insertion locus. The interpretation of such data will, however, be challenging, as a greater understanding of gene functions and changes in expression levels is required before the safety implications of any such change in gene expression can be assessed.

Assessment of unintended effects

20. Uncertainties in the safety assessment associated with unintended changes in plant genomes through the insertion of recombinant DNA should always be considered in the light that crop genomes are constantly changing through a broad range of natural and man-mediated mechanisms. Uncertainty associated with food safety of GM crops is no greater than uncertainty associated with conventionally bred crops. Unintended effects that alter the composition of food crops are as likely to occur through natural recombination and mutagenesis approaches used in plant breeding as through genetic modification. Variety selection and registration requirements for both GM crops and conventionally bred counterparts that involve the assessment of physiology, morphology, and performance are sound indicators of unintended effects that may potentially impact human health.

21. The application of profiling techniques providing fingerprints of a crop samples' gene expression profile, protein levels, and metabolite levels promises to rapidly expand our understanding of metabolic and compositional variations of crop plants. Profiling methods are, however, not suitable as yet as a commonly used tool for the safety assessment of GM crops. Genomic research and new tools of molecular biology such as high-throughput DNA detection and characterisation methods will also greatly increase our understanding of crops and their genomes, their interaction with their environment, and implications to health from their consumption. *In vitro* and *in vivo* test systems using genomic and micro-array technologies in order to provide sensitive biomarkers for biological responses to food components and possibly whole foods may in future allow more specific prediction of which health endpoints are affected by specific toxins, by improving knowledge of human metabolic and cellular processes and by having the possibility of monitoring subtle changes in gene expression levels. Further development of these methods is encouraged.

22. Several issues need to be addressed before profiling approaches can become a proven and useful tool in standard risk assessment procedures. The interpretation of outputs (data) remains a significant challenge. Much work remains to be done in the development and standardisation of sampling procedures and approaches for data collection and handling. Inter-laboratory "ring" testing and validation of these methods will also be required. In addition, a more comprehensive understanding of natural variation in, for example, the levels of metabolites in crops needs to be developed to allow any unintended changes in a GM crop plant to be properly "benchmarked". Most importantly, approaches will be needed to interpret the biological relevance and toxicological significance of any observed differences. The identification of possible differences between traditionally used and novel crops that might have adverse effects on human health is the overall aim of both targeted and non-targeted approaches.

23. ENTRANSFOOD recommends international research efforts for the development of profiling methods and international databases on natural variation in gene expression, protein, and chemical composition of crops. The allocation of public sector research funds to this aim is important. These methods will contribute to improving our understanding of the foods we eat and their potential implications for human health.

Assessment of risks of gene transfer across species

24. Gene transfer between organisms is common in nature and has been a driving force in evolution. There is no inherent risk in the transfer of DNA between organisms, since DNA is not toxic. The risk of gene transfer of recombinant DNA from GM crops to microbes or human cells has to be evaluated with respect to the risk of a similar event occurring in nature. The potential impact largely depends on two factors: first, on the function of the transferred DNA in the recipient cell; and secondly on whether the recipient cell may have acquired the same gene from a source other than the GM crop.

25. The risks of gene transfer from GM crops that are currently commercial are deemed negligible. Transfer to microbes by transformation is a possibility, but only consequential if a new trait is expressed and confers selective advantage. Uptake of GM crop-derived DNA, including the transgenes by human cells of the gut or the immune system, cannot be ruled out; it is, however, very unlikely that transgenic DNA is stably integrated in somatic cells or taken up in germline cells. Even if it should be taken up, the trait conferred by the gene may not be expressed in human cells.

26. The risk of use of antibiotic resistance markers for selection of transformed plant cells should be judged on a case-by-case basis, considering their frequency of occurrence in bacterial populations and the extent of clinical use of the antibiotics to which resistance is conferred (and whether the antibiotic is of importance as a last resort). Some antibiotic resistance markers such as the *nptII* gene and the hygromycin resistance gene can be used without the risk of compromising the use of important clinically used antibiotics.

27. The transformation strategy and the recombinant DNA for insertion into the GM crop should be designed with care. Guidelines on best practice recommend minimising recombinant DNA sequences transferred to GM crops in order to simplify the molecular characterisation and reduce uncertainty on potential genetic rearrangements and unstable gene expression. *Agrobacterium*-mediated gene delivery is considered the most controlled gene delivery system that facilitates obtaining GM crops with single, simple and hence minimal inserts.

The design of recombinant DNA transferred to GM crops should minimise the risks of DNA sequences that might foster independent transfer, expression of the DNA in microbes or viruses, and recombination in bacterial or viral genomes, where possible.

28. Further research and development on transformation methods and methods for the elimination or replacement of selectable markers is encouraged, in particular in the public sector. Public access to such methods is important. Improvements in methods for crop transformation aim at simplifying the safety assessment of GM crops by reducing the introduced recombinant DNA in GM crops to a minimum. The risks, costs, and benefits of established and potential new transformation technologies should however be systematically compared before novel technologies are officially recommended for broad adoption in product development.

Detection, labelling, and traceability of GMOs

29. The General Food Law provides an integrated approach to ensuring food safety across the EU Member States and across the food and feed sectors. The General Food Law provides for one decision-making procedure for all food products that require EU-level approvals, such as food additives, pesticide residues in food, novel foods, and genetically modified organisms (GMOs). The European Commission Directorate General for Health and Consumer Protection administers the decision process. The European Food Safety Authority reviews the risk assessment submitted by applicants intending to place food additives, pesticides, novel foods or foods derived from GMOs on the European market. The law also clarifies accountability of all legal entities involved in food production and regulation in the EU by describing general food safety requirements that are imposed on both the Member States and business operators. General principles in the law include the protection and information of consumers through comprehensive labelling schemes; provisions for traceability, that is the ability to trace back to the origin and to understand the distribution of foods and food ingredients; and the application of the precautionary principle in instances of significant uncertainty in the risk assessment.

30. In June 2003, the European Council of Ministers adopted two new Regulations specific for foods and feeds derived from GMOs. Regulation (EC) No 1829/2003 on GM food and feed provides the legal basis for the approval procedure for GMOs as specified in the General Food Law. The European Food Safety Authority's Scientific Expert Panel on Genetically Modified Organisms assesses the safety of foods derived from GMOs. The panel also assesses the food safety, environmental, and animal health aspects of GMOs ("one-door-one-key" principle).

31. Regulation (EC) No 1830/2003 concerning the traceability and labelling of GMOs and the traceability of food and feed products produced from GMOs requires labelling of all food products derived from GMOs, including those that do not contain detectable traces of recombinant DNA or novel protein such as highly refined oils (not materially distinguishable from other oils not derived from GMOs).

32. ENTRANSFOOD considers that current thresholds for labelling requirements for foods containing or consisting of GM crops are very stringent and pose challenges for implementation. The threshold of GM crop content above which a food needs to be labelled is 0.9% for GM crops that have been approved for import or cultivation in the EU and 0.5% for a GM crop reviewed by an EU scientific committee, but that has not been as yet approved. Given the significant administrative burden from the implementation of current labelling

legislation and standards on governments and producers, ENTRANSFOOD recommends to develop diversified sampling plans, more stringent plans for those cases where the safety of the food supply is at stake, and more lenient sampling regimes for other cases. Clear rationales for labelling of products should drive the determination of such standards. Closer collaboration between consumer groups, scientists, and legislators, in particular in standard setting, is required to ensure agreement on standards that can be implemented and enforced.

33. Prerequisites for the implementation of the new labelling and traceability provisions include the establishment of systems for documenting the distribution of individual GM crops in the agro-food chain and analytical methods for verification of this information. Sampling and detection methods for verification of presence or absence of GM crops require qualitative and quantitative approaches. Qualitative detection methods are required for high-throughput screening of large quantities of samples or distinguishing approved from non-approved GM crops. Development of such methods requires reference materials and product information for all individually transformed GM crops that are in development or commercialised anywhere in the world. The European Network of GMO Laboratories was set up for this purpose; a similar system, however, needs to operate globally. Guidelines are required outlining detailed standards for the purity and type of reference materials and additional information on the GM crop that needs to be provided.

34. Sampling plans depend on the quality and nature of the detection method that is used and on the threshold set. Appropriate sampling schemes for bulk loads where GMO-derived materials may be mixed in a very heterogeneous way will require the analysis of large numbers of samples per load. Reliability and costs of sampling and testing depend very much on the test material in terms of the non-uniformity of distribution of GMO-derived material in the sample and the 'food matrix' from which DNA or the protein have to be extracted for detection. Given the burden on administrations and producers, ENTRANSFOOD recommends developing diversified sampling plans, more stringent plans for those cases where the safety of the food supply is at stake, and more lenient sampling regimes for other cases.

35. The three specific objectives of legal provisions for traceability are to facilitate withdrawal of products should an unforeseen risk to human health or the environment be established; targeted monitoring of potential effects on human health or the environment, where appropriate; and control and verification of labelling claims. The fundamental objective is to restore consumer trust through providing information and choice. Traceability of foods and food ingredients, including imported foods, also requires, however, the establishment of international systems allowing traceability of traded foods.

36. ENTRANSFOOD recommends considering whether additional testing for verification of claims on GM crop content should not only be carried out at critical control points in the food supply chain. In some cases, paper-based or electronic traceability systems may be adequate.

37. At present, the lack of tools for detection of diverse GM crops released into the environment poses significant challenges for the food industry to comply with EU labelling legislation. Enforcement of the law is equally difficult. Furthermore, EU law requires separate registration of seeds in which two different traits obtained by genetic modification (such as insect resistance and herbicide tolerance) have been combined by breeding, often called 'stacked genes'. Enforcement of this requirement would also require detection methods that can distinguish between two traits that were stacked into one seed by breeding and two seeds of which each seed contains one trait. In the analysis of commodity shipments that may

contain both types of seeds (single trait and stacked trait seeds), drawing such distinctions is impossible with current detection methods.

38. Traceability represents a valuable tool to face problems related to the introduction of GMOs in food and feed chain and to gain the confidence of the consumer toward this novel food. It is clear, however, that the implementation of any suitable system implies a substantial increase in overall cost of food production that will have to be absorbed by both producers and consumers. This holds true unless a traceability system also helps to save on current costs, such as their use as a tool for more effective inventory- and supply chain-management.

Societal aspects of introducing GM crops in the agro-food chain

39. The public framing of questions on risk often differs from the framing of scientists. For instance, there is a concern on potential long-term effects from adopting GM crops. Whether society 'needs' the technology is also considered important. If public confidence in the technology and its regulation is to be regained, it is important to explicitly incorporate public concerns into the risk analysis process through developing new and influential methods of stakeholder involvement and consultation (including consumers). Once public concerns and the values on which they are based are understood, they can be more effectively introduced into innovation strategies, risk assessment, and risk management practices.

40. Surveys conducted in this project supported that consumers may prefer labelling of products on the basis of both process and product characteristics. Research is needed to determine the most effective form for food labels, which take due account of cross-cultural differences in information preferences where they exist.

41. Questions for further research on societal aspects of GM foods include the following: How can public concerns be incorporated into this process? How can effective and inclusive public participation in risk management and science and technology policy be developed? What is the best way to involve the public in the debate about genetic modification of foods? How might information about the difference this has made to public policy be communicated back to the public? What changes to institutions need to be made in order to accommodate these processes? How should they restructure themselves to make it easier for the voice of the public to be heard?

The need for an integrated platform for deliberation on food production and biotechnology

42. The ENTRANSFOOD project has highlighted the need to continue interdisciplinary deliberations on agricultural biotechnology to better understand potential impacts from adoption and diverse approaches to regulation of the technology. Four types of questions should be considered more systematically, integrating a wide range of different perspectives: what is the objective of a new product or technology for food production, who will benefit from it, who might incur risks (environmentally, health-wise, economically, or culturally), and how can we ensure that we will learn from the experience?

43. ENTRANSFOOD recommends the establishment of a Permanent Evaluation and Discussion Platform that explores both scientific and societal issues of diverse current practices in food production, i.e. intensive agricultural production, low-input/organic production, and genetic modification-facilitated production practices. Regulators, academics, and stakeholders from the private sector and consumer organisations should work together to map areas where there is agreement, disagreement, and the need for further research. Such a Platform could have

several functions, such as organising events to frame questions on risk for expert deliberations, guide the assembly of the knowledge base for experts, review draft expert advice, and review proposed draft regulations and standards. Deliberations on how institutions can continually improve their approaches to governing risks from biotechnology could also be part of the remit of such a platform. Coordinators of such an integrated deliberation platform should also consider on how they should interact with intergovernmental organisations working on guidelines and policy recommendations on agro-food production and biotechnology, such as the Codex Alimentarius, the Organisation for Economic Coordination and Development, and Organisations of the United Nations.

List of abbreviations

ADI, acceptable daily intake
BSE, bovine spongiform encephalopathy
cDNA, DNA complementary to an RNA strand
CEN, Centre Européen de Normalisation
DG SANCO, European Commission Directorate General for Health and Consumer Protection
DNA, deoxyribonucleic acid
EC, European Commission
EEC, European Economic Community
EFSA, European Food Safety Authority
ENTRANSFOOD, European network safety assessment of genetically modified food crops
EU, European Union
FAO, Food and Agricultural Organisation of the United Nations
FOSIE, EU Concerted Action on Food Safety in Europe
FSA, British Food Standards Agency
GI, gastrointestinal
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 sciences facing the multiplicity of new released GMO, measurement and testing
HACCP, Hazard analysis critical control points
mRNA, messenger RNA
ISO, International Standards Organisation
NGO, non-governmental organisation
NOAEL, no observed adverse effect level
OECD, Organisation for Economic Cooperation and Development
PCR, polymerase chain reaction
Qpcrgmofood, EU-project on reliable, standardised, specific, quantitative detection of genetically modified foods
SAFOTEST, EU-project about new methods for the safety testing of transgenic food
R&D, research and development
RNA, ribonucleic acid
RTD, research and technology development
T-DNA, transfer DNA
Ti, tumour inducing
UK, United Kingdom
US, United States
WHO, World Health Organisation

INTRODUCTION

Worldwide, the consumption of foods derived from genetically modified crops (GM crops) is rising rapidly. Since the first GM crop was introduced in the market for large-scale cultivation in 1996, the global area of cultivation has risen to 67.7 million hectares in 2003 (Figure In-1; James, 2003). An increasing number of countries approved an increasingly diverse array of crops with new traits. A large share of the harvest enters the agro-food chain. In 2002, three crops largely shared the total global acreage of GM crops: GM soybeans, maize, cotton, and oilseed rape accounted for 61%, 23%, 11%, and 5%, respectively. The rate of adoption varied across countries: plantings in the US, Argentina, and Canada accounted for 63%, 21%, and 6% of the total global area of cultivation; in Europe, GM crops were grown in Romania (70,000 hectares of GM soybean), Spain (32,000 hectares of insect-protected maize), Germany, and Bulgaria. The first GM crops that are currently marketed have been genetically modified to facilitate cultivation and to reduce the environmental impact of agriculture. These crops contain new genes that protect the crop against certain insect pests, or that confer tolerance to broad-spectrum herbicides facilitating weed control. GM crops with more complex modifications that affect a crop's metabolism or physiological processes, such as the enhancement of properties relating to a crop's food safety or nutritional characteristics. For instance, rapeseed with higher levels of vitamin E and rice containing pro-vitamin A and/or iron are in development, and research is being conducted to lower the levels of natural allergens in rice and peanuts. The removal of other natural toxins is also being considered. Regulatory authorities are considering some such crops for approval, as for example soybeans and rapeseed with altered fatty acid composition.

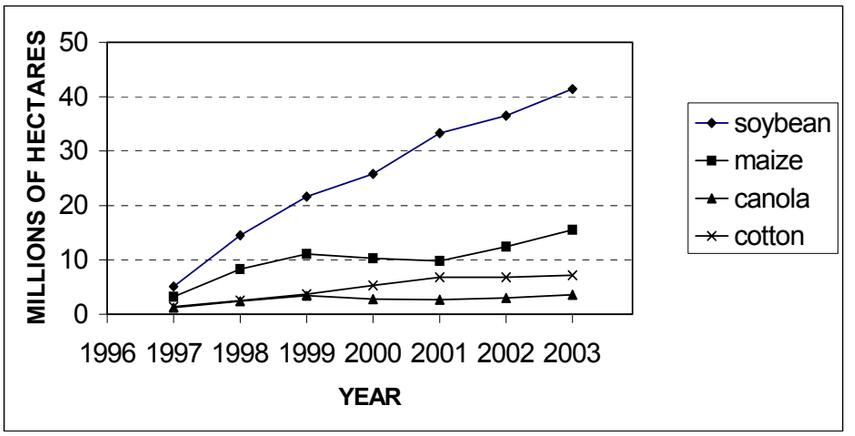

Figure In-1 Global cultivation of GM crops (James, C., 2003. Global Status of Commercialized Transgenic Crops: 2003, ISAAA Briefs No. 30: Preview. International Service for the Acquisition of Agri-biotech Applications, Ithaca)

The Thematic Network ENTRANSFOOD provided a platform for deliberation on the interdependent scientific, regulatory, and societal aspects of food safety assessment of GM crops by a multidisciplinary group of researchers and administrators. The underlying premise of deliberations on the food safety of GM crops under the auspices of ENTRANSFOOD has been the comparison of the safety of food crops produced by conventional means to foods derived from GM crops.

Alterations of plant genomes at the molecular level occur through similar mechanisms in plant breeding and the application of methods of agricultural biotechnology, and hence are thought to occur even in similar regions of plant genomes; what differs is the range of source organisms from which new genetic variation can be introduced. Plant breeding relies on systematically identifying beneficial changes in crops resulting from natural genetic variation and on selecting these improved varieties for further propagation and finally cultivation. Breeders have also used artificial means to enhance genetic variation; these include the use of chemical or gamma irradiation mutagenesis to increase the error rate in DNA replication processes in plant breeding. The extent to which DNA structure and integrity are modified in these accepted breeding approaches is unknown. What is clear is that the genetic structure of plant populations has been widely changed by breeding practices, indicating the deep influence of breeding practices on the genetic make-up of plant crops. Genetic engineering of crop plants largely relies on two methods used to introduce foreign DNA into plant cells: biolistic (microprojectile) bombardment and *Agrobacterium*-mediated transformation. The biolistic method is based on a physical delivery of DNA-coated gold or tungsten microprojectiles into plant target tissue by acceleration. *Agrobacterium*-mediated transformation exploits the biological ability of this soil-borne bacterium to copy and transfer a specific portion of DNA (termed T-DNA) present on a tumour inducing (Ti) plasmid into the plant cell nucleus, where it can be integrated into chromosomes.

Scientists rely on the same fundamental approach to assess the safety of individual GM crops. Each individual GM crop is compared to an appropriate comparator that is generally accepted as safe for food use, usually based on its extensive prior use as a human food. The much slower adoption of the technology in Europe, however, demonstrates that rigorous safety assessment is necessary but not sufficient for gaining societal acceptance of agricultural applications of biotechnology.

Regulatory frameworks and decisions on prerequisites for the safe and sustainable adoption of the technology differ across jurisdictions. The United States amended existing product legislation including the Federal Plant Pest Act and the Food, Drug and Cosmetics Act to regulate GM crops. Over the last nine years, the United States regulatory agencies have permitted commercial cultivation of over 50 different GM crops products. In the European Union, new legislation was drawn up specifically for the regulation of genetically modified organisms (GMOs). Only thirteen GM crops have been approved for environmental release before October 1998, and none since then. In June 1999, five Member States declared that new authorisations for the environmental release of GM crops shall be suspended until a more rigorous and transparent regulatory framework is adopted that requires environmental monitoring where appropriate, monitoring, and the labelling and traceability of all foods derived from GM crops. Thirteen GM crop-derived processed food products are approved for consumption in the EU.

Consumer and environmental organisations have challenged official risk assessment and risk management procedures, largely on the ground that uncertainties on long-term effects of GM food crops on both health and the environment are not adequately addressed. The need to ensure that consumers can choose whether to consume foods derived from GM crops through labelling and traceability requirements was emphasised. Regulatory institutions and the industrial players attempting to commercialise products have now taken measures they perceive to be in the public's interest. In particular since the series of food scares in Europe culminating with BSE, trust in Europe's food safety system (assessment methods, legislation, and institutions) needs to be rebuilt. European Commission officials hope that the recent adoption of a new regulation on foods and feeds derived from GMOs and a regulation on the labelling and traceability of foods

derived from GM crops by the Council will provide an appropriate basis for Member States to resume the regulatory process for decisions on individual products.

Introducing a new technology in society requires awareness of the interests and values stakeholders associate with the technology and repercussions from technology adoption on each. The agro-food chain is complex, consisting of elements with diverse interests, including farmers, traders, distributors, processors, retailers, and end-consumers. Food processors and retailers are often more sensitive to concerns of the end-consumer than organisations at the natural resource extraction-end of the value chain (such as Agbiotech providers and seed companies). Furthermore, interests of end-consumers and entities in the agro-food chain closest to them (retailers and food processors) may be different in diverse markets.

This report, a synthesis of deliberations of the food safety of GM crops of five Working Groups with members from diverse backgrounds, is divided into seven chapters. Chapter 1 provides an introductory overview on food safety regulation of GM crops and international guidelines for risk analysis and safety assessment of GM crops. The subsequent five chapters summarise outcomes of deliberations of ENTRANSFOOD's five working groups. The five working groups each covered the following topics: safety assessment; unintended effects; horizontal gene transfer; detection, labelling and traceability; and societal aspects. Chapter 2 describes how to tailor safety assessment strategies for specific GM crops in a systematic and stepwise manner. Chapter 3 outlines how to characterise and reduce uncertainties in the detection of unexpected effects possibly due to the genetic modification. Considerations on whether there may be risks inherent in GM crops that are not associated with crops improved through conventional breeding technologies were central to the deliberations. Chapter 4 describes the potential risks from the transfer of recombinant DNA from GM crops to microbes or human cells. Chapter 5 identifies scientific, methodological, and institutional prerequisites for the implementation of proposed legislation on labelling and traceability. Chapter 6 considers what influences public attitudes to the technology, and trust in institutions and information. Chapter 7 presents conclusions, discussing the limitations and successes of this project.

ENTRANSFOOD is a successful example of how issues of food safety assessment, uncertainties, regulation, and societal impacts can be elucidated from a range of different perspectives, including from scientists, regulators, firms, and civil society organisations. One limitation discussed in more detail in Chapter 7 stems from the project's focus on food safety and attitudes to agricultural biotechnology in Europe. The scope precluded more detailed consideration of attitudes to and impacts from adoption of alternative farming practices comparing organic farming practices, conventional approaches relying on use of agro-chemicals, and use of GM crops in Europe or elsewhere in the developed or developing world. Questions on distributional effects from regulation, and liability and insurability of risks, including risks from regulations that are difficult to comply with, also were beyond the scope of ENTRANSFOOD. Detailed consideration of ethical issues and assessment of the impact of adoption of the technology on diverse interest groups and stakeholders were, however, beyond the scope of this work. These aspects, central to a more comprehensive assessment, need to be considered in future research projects. Although we could not address them in more detail, we consider all the above questions central to society's debate on the future of agricultural biotechnology.

The ENTRANSFOOD project has highlighted the need to continue interdisciplinary deliberations on agricultural biotechnology to better understand potential impacts from adoption of the technology from diverse perspectives of groups defending distinct sets of values. Four types of questions should be considered more systematically, integrating a wide range of different perspectives: what is the objective of a new product or technology, who will benefit from it, who

might incur risks (environmentally, health-wise, economically, or culturally), and how can we ensure that we will learn from the experience? Chapter 7 presents conclusions and recommendations for future research and a proposal to establish an improved integrated discussion platform for general deliberations on food production and biotechnology, building on experience gained in ENTRANSFOOD.

CHAPTER 1

REGULATION AND RISK ANALYSIS OF FOODS DERIVED FROM GM CROPS: AN OVERVIEW

Food safety analysis and control systems, comprising institutions, policies, laws, and guidelines for assessments, continually evolve over time. The evolution of such systems in individual jurisdictions is affected both by science and society: Scientific advances improve our understanding of health implications of foods and lead to the development of new foods that might require regulatory oversight. Changing societal values and norms can lead to shifts in emphasis in consumer protection policies and regulatory and institutional change. Regulation in turn can affect both innovation and risk perception.

Regulatory frameworks differ across jurisdictions. The European Union (EU) regulatory system is representative of this *"process-based"* approach. Separate legislation for the environmental release and the food and feed derived from GM crops was created. In other legal systems, such as the US, existing product legislation is amended to apply to GM crop-derived foods. The US Federal Food Drug and Cosmetics Act, the scope of which was broadened to include foods derived from GMOs, is an example of vertical, *"product-based"* regulation. Regulatory decisions on product approvals and regulatory prerequisites for the sustainable deployment of agricultural biotechnology differ: the US has continued to approve GM crops for commercial cultivation; the EU has instituted a *de facto* moratorium since 1998. The EU advocates establishing a system for process-based labelling and traceability of foods derived from GM crops as a prerequisite for GM crops entering the global food chain. Whilst regulatory frameworks and decisions differ across jurisdictions, the same approach to the safety assessment of foods derived from GM crops was adopted in most countries. The approach is based on general principles for risk analysis and international guidelines for the safety assessment of foods derived from GMOs.

This chapter provides an overview on the regulation of foods and GM crops in the EU. Subsequently it describes general concepts of risk analysis, food safety assessment, and international guidelines on how to apply the principles of risk analysis and food safety assessment to the assessment of foods derived from GM crops.

1.1 Regulation of food safety and of GM crops in the European Union

In the 1980s, the main motivation for establishing a common food policy amongst Member States of the European Economic Community was the creation of the single market; this required harmonising Member State standards and labelling schemes that might hinder trade if too distinct. The early cautious approach to harmonisation of food law of the EU Member States resulted in a large number of fragmented laws, which include horizontal framework directives with general provisions on food additives, labelling, and hygiene of foods, and vertical product-specific directives, such as those with hygiene provisions for specific animal-derived food products. Overall, the main objective of helping to establish the single market was achieved, but the resulting set of directives was often criticised as opaque, fragmented, incoherent, and as in general not adding any benefit to existing EU Member State legislation. The late 1980s and early 1990s also saw the implementation of directives for the regulation of food additives, pesticide residues in foods, and contaminants in foods.

Before 1997, foods and feeds containing GMOs were approved through Directive 90/220/EEC on the deliberate release and placing on the market of GMOs. The directive, administered by Directorate General for the Environment, regulates the deliberate release of all live GMOs regardless of their field of application. Only the placing on the market of GMOs with medical

uses, such as live vaccines, and of foods containing or consisting of GMOs are exempt as regulated elsewhere. Two GM crops were approved for import and food use under this directive: Monsanto's herbicide-tolerant soybeans and Syngenta's insect-protected corn (developed by Ciba Geigy). In June 1999, five member States (Denmark, France, Greece, Italy, and Luxembourg) declared: "in accordance with the precautionary principle, new marketing authorizations shall be suspended". The declaration called for the adoption of a more rigorous and transparent regulatory framework that, among other improvements, sets out provisions for monitoring requirements of transgenic crops. The consequence, although not officially declared, is a *de facto* moratorium on the commercial cultivation of transgenic crops in the EU (see for lists of approved or pending products, European Commission, 2004).

Later in the 1990s, the Bovine Spongiform Encephalopathy (BSE) crisis was the single most important trigger for regulatory reform of the current food safety analysis and control systems in the European Union. First, it highlighted the need for improved coordination in the adoption of risk management measures in the area of food safety across the Member States. The BSE crisis and the spread of dioxin-contaminated feed batches also highlighted the need for a vertically integrated approach to the governance of feed and food safety that addresses all stages of production 'from farm to table'. The crises also undermined consumer confidence in Europe's food safety systems.

Institutional and legislative changes ensued. Institutional changes included the creation of the European Commission Directorate General for Health and Consumer Protection (DG SANCO), in 1997, and the creation of the European Food Safety Authority (EFSA) in 2002. Legislative changes include the final agreement between Member States, the Commission, Parliament, and the Council on the long negotiated Regulation (EC) No 258/97 concerning novel foods and food ingredients (henceforth Novel Foods Regulation). The Novel Foods Regulation gave the European Commission a clear role in the governance of food safety. This role was strengthened with the publication of the Regulation (EC) No 178/2002 on the general principles of food law and the establishment of the European Food Safety Authority (hence forth the General Food Law).

The Novel Foods Regulation provides an EU-wide regulatory approval system for novel foods that "have not hitherto been consumed to a significant degree in the EU". This includes foods derived from GMOs. Each EU Member State appointed a national competent authority for administration of the law at the national level. The regulation is at present being revised in order to be adapted to provisions of the 2002 General Food Law. The Novel Foods Regulation, which entered into force in 1997, provided for two alternative decision-making procedures for the placing on the market of foods derived from or containing GM organisms. The determination of "substantial equivalence" of the novel food to an appropriate comparator with an accepted standard of safety by the rapporteur Member State serves as regulatory clearance for marketing of the product and is notified to the European Commission. Processed oil from GM oil seed rape and cotton, and processed products derived from GM corn are amongst the products notified based on a determination of substantial equivalence under the Novel Foods Regulation. For lists of approved or pending products see European Commission (2004). Novel foods that are not deemed substantially equivalent needed to undergo a more complex authorisation procedure involving a review by all EU Member States. One ambiguity under the Novel Foods Regulation relates to the definition of the term substantial equivalence that determines the choice between the two distinct procedural options for marketing foods.

The 2002 General Food Law now provides an integrated approach to ensuring food safety across the EU Member States and across the food and feed sectors. General principles in the law state

that risk analysis is based on scientific risk assessment conducted by the recently instituted European Food Safety Authority (EFSA). The law establishes an EU-level authorisation procedure for all types of regulated foods. Other general principles include the protection and information of consumers through comprehensive labelling schemes; provisions for traceability, that is the ability to trace back to the origin and to understand the distribution of foods and food ingredients; and the application of the precautionary principle in instances of significant uncertainty in the risk assessment. Furthermore, the new law clarifies accountability of all legal entities involved in food production and regulation in the EU by describing general food safety requirements that are imposed on both the Member States and business operators.

The primary responsibility of compliance with the EU food law is placed on business operators at all stages of the producing, processing, manufacturing, or distributing of food. This is to be achieved through implementation of self-checking provisions relying on the Hazard Analysis Critical Control Points systems (HACCP). The Member State competent authorities are to monitor and verify compliance of private sector entities with the law. The law also provides a legal basis to the Commission's coordination of an EU-wide rapid alert and crisis management system network of national institutes and competent authorities, in which DG SANCO plays an important role.

Furthermore, the General Food Law provides for one decision-making procedure for all products that require EU-level approvals, such as food additives, pesticide residues in food, novel foods, and GMOs. The procedure is as follows: the European Commission DG SANCO administers the review process. As provided for in the General Food Law, EFSA reviews the risk assessment submitted by applicants intending to place a novel food on the European market. It is up to the administrators in European Commission DG SANCO to draft proposals based on the risk assessment and other broader considerations that may affect choice of policy options. A regulatory committee of representatives of Member States competent authorities then decide whether to accept the Commission proposal through a weighted voting system. If the regulatory committee's opinion is not in accordance with the proposed measure or if no opinion is delivered, the question is referred to the Council of Ministers. The Council of Ministers can approve or reject a Commission proposal given a qualified majority of Member States support the position. If rejected, the European Commission has to prepare a new proposal. If the Council of Ministers takes no decision within three months, or does not reach a qualified majority indicating that it opposes the proposal, the European Commission shall adopt the proposal.

Regulation of GMOs

Currently, Directive 2001/18/EC on the deliberate release and placing on the market of GMOs govern environmental releases of GM crops. It repeals the former Directive 90/220/EEC. Unlike in the US, lines of GM crops that contain two traits that have previously been registered in separate crop lines, but that are combined in one crop through breeding require separate registration. The revised directive strengthens the existing rules of the risk assessment and the decision-making process on the release of GMOs into the environment. In particular, it defines mandatory information that must be given to the public and introduces general rules on mandatory labelling and traceability at all stages of the placing on the market. Authorisations will be granted for a period of 10 years, subject where appropriate to a post-market monitoring plan.

The Regulation (EC) No 1829/2003 on food and feed derived from GMOs was adopted in July 2003. There is one single authorisation procedure for the food use, feed use, and commercial release of GMOs that corresponds to the procedures described in the General Food Law (see also

Box 1-1). This 'one-door-one key' regulatory strategy is in line with the European Commission's effort to integrate food and feed regulation across sectors and to enhance the efficiency and coherence of the review system. It is also hoped that this strategy will help to avoid fiascos such as the finding of Aventis' Starlink™ insect-protected corn in the food chain, although it had only been approved for local cultivation and feed use in the US.

BOX 1-1

EU LEGISLATION ON GM FOODS

Environmental release
Directive 2001/18/EC on the deliberate release into the environment of GMOs and repealing Council Directive 90/220/EEC

GM food and feed
Regulation (EC) No 1829/2003 on GM food and feed

Contained use of GM micro-organisms
Council Directive 90/219/EEC on the contained use of GM micro-organisms
Council Directive 98/81/EC amending Directive 90/219/EEC on the contained use of GM micro-organisms

Labelling of GM food and feed
Council Regulation (EC) No 1139/98 concerning the compulsory indication of the labelling of certain foodstuffs produced from GMOs of particulars other than those provided for in Directive 79/112/EEC
Commission Regulation (EC) No 49/2000 amending Council Regulation (EC) No 1139/98 concerning the compulsory indication on the labelling of certain foodstuffs produced from GMOs of particulars other than those provided for in Directive 79/112/EEC
Commission Regulation (EC) No 50/2000 on the labelling of foodstuffs and food ingredients containing additives and flavourings that have been genetically modified or have been produced from GMOs
Regulation (EC) No 1830/2003 concerning the traceability and labelling of GMOs and the traceability of food and feed products produced from GMOs and amending Directive 2001/18/EC

Labelling, traceability, and consumer choice

The European Commission's role in consumer protection has grown together with its increasingly political remit since entry into force in 1987 of the Single European Act. This priority has been further highlighted in the Treaty of Amsterdam. The website of the European Commission DG SANCO spells out that provision of consumer information and choice through labelling and product information is considered an important element required to re-establish consumer trust in the EU food safety analysis and control system after the BSE and other food safety crises. Information should be provided on health-related issues and on salient matters related to food production processes.

In the 1970s, European Community law to harmonise Member State approaches to labelling, presentation, and advertising of foodstuffs were established to protect from unfair competition (different label provisions across Member States prevented free movement of goods) and to protect and provide information to the consumer. The resulting Directive 79/112/EEC on labelling is complemented by some sectoral product-specific requirements in EU law relating to hygiene or quality requirements for specific food products. The labelling provisions were recently consolidated and simplified by Directive 2000/13/EC on the labelling, presentation, and advertising of foodstuffs. The Directive applies to pre-packaged foodstuffs to be delivered as such to the ultimate consumer or to restaurants, hospitals, canteens, and other similar mass caterers. It does not apply to products intended for export outside the Community. Labelling provisions in the General Food Law are that labelling and product presentation should allow consumers to make informed choices and that consumers shall not be misled.

The 1997 Novel Foods Regulation's Article 8 provides that food and food ingredients that are deemed to be no 'longer equivalent' to existing products need to be labelled for consumer information purposes, additionally to health, safety, and nutritional considerations. This labelling policy provides for consumer information on changes in composition due to production processes, even if such changes may not impact human health. Specific labelling is not required where recombinant DNA and/or protein derived from the genetic modification is present in the food or food ingredient in a proportion no higher than 1%, provided there is evidence that this presence is not intended but occurred inadvertently during cultivation, harvest, transport, storage and processing (Regulation (EC) No 49/2002). These provisions also applied to the two GM crops in foods approved before the Novel Foods Regulation entered into force: Bt-176 maize and Roundup Ready™ soybean.

In July 2003, the Council of Ministers adopted Regulation (EC) No 1830/2003 concerning traceability and labelling of GMOs and traceability of food and feed products produced from GMOs. According to the regulation, labelling shall be process-based: even highly refined oils that do not contain detectable traces of recombinant DNA or novel protein from GMOs, and hence are not materially distinguishable from other oils not derived from GMOs will need to be labelled. The three specific objectives of legal provisions for traceability are to facilitate withdrawal of products should an unforeseen risk to human health or the environment be established; targeted monitoring of potential effects on human health or the environment, where appropriate; and control and verification of labelling claims. The fundamental objective is to restore consumer trust through providing information and choice. Traceability of foods and food ingredients, including imported foods, also requires, however, the establishment of international systems allowing traceability of traded foods.

Establishment of an international system that allows to trace back to the origin and to understand the distribution of foods would depend on agreement on at least three of the system's elements: each product must have a unique identifier (a bar code, lot identification number, or container identification marking in case of commodities); guidance must be given on what specific information is recorded; and all points in the production and distribution chain at which this information is recorded must be reliably linked. Audits for verification of the implementation of the system are also required.

Transparency and participation

Over the last decade a series of more general calls for openness and participation have been introduced into primary and secondary European Community law. Article 1 of the Treaty of the European Union establishes as a general principle that decisions are to be taken as openly as

possible and "as closely as possible to the citizen". There are no or few legal obligations that specify concrete procedural mechanisms to foster transparency and participation, other than that the provisions of scientific committees of the European Commission have to be posted on the internet; this also holds true for the majority of the individual decision-processes in the various Member States. The few procedural windows are, however, a modest contribution towards institutional transparency: the value to the citizen of internet postings of expert opinions is very limited, as there are usually no links to data considered by experts, explanations of the relevant EU-level decision-making processes, and the role of expert opinions in it. In conclusion, insufficient light is shed on such processes to allow an average citizen to understand or even to contribute to them. In consequence, consultation with stakeholders, if it occurs, is a lot less formal and transparent than in the US, since there are no formal procedures based in law in place to ensure that 'all' viewpoints are considered. The Scientific Steering Committee of the European Commission has also recommended seeking processes to foster public engagement in the process of risk assessment (European Commission, 2003).

1.2 Risk analysis of foods

The general principles for risk analysis were first established for evaluation of health effects from potentially toxic chemicals. Risk is defined as the likelihood that, under particular conditions of exposure, an intrinsic hazard will represent a threat to human health. Risk is a function of hazard and exposure. Hazard is defined as the intrinsic *potential* of a material to cause adverse health effects; implicit in the definition is the concept of severity and adversity of the effect (FAO/WHO, 1995; FAO/WHO, 1997; Codex Alimentarius Commission, 2003).

In most international and European policy documents and guidelines on risk analysis and food safety (see for example European Commission, 2000), a distinction is drawn between science-based risk assessment (usually conducted by experts), risk management, and risk communication. Risk management is defined as "the process of weighing policy alternatives to mitigate risks in the light of risk assessment and, if required, selecting and implementing appropriate control options, including regulatory measures" (FAO/WHO, 1995; FAO/WHO, 1997). Risk management strategies include authorisation, and implementation of risk management measures to minimise the risk. Examples of risk management for conditional approvals include labelling requirements to inform the target group at risk, as done for food products that contain major allergens. Risk communication is defined as the exchange of information and opinion on risk between risk assessors, risk managers, other interested parties, and the general public (FAO/WHO, 1995; FAO/WHO, 1997). Recently, the European Commission has undertaken an initiative for harmonisation of risk assessment procedures (European Commission, 2003b). In Figure 1-1, the various components of risk analysis are depicted as described in the report of this initiative. The report further recommends to involve stakeholders in two stages of the risk analysis process, namely before- and after- the risk assessment process. In this way, the risk issues that are important to stakeholders can be taken into account and these stakeholders can also comment on the outcome of the risk assessment (European Commission, 2003b).

Some social scientists warn that considering risk assessment and risk management as two separate processes in policy-making can blindfold to the notion that risk is also a product of societal circumstances; the salience of expert advice to concerns of policy makers and the public is thus potentially reduced (Jasanoff, 1990; NRC, 1994; NRC, 1996; Presidential/Congressional Commission on Risk Assessment and Risk Management, 1997). The importance of framing questions on risk such that assessments address socially salient concerns has been much emphasised, see Figure 1-2).

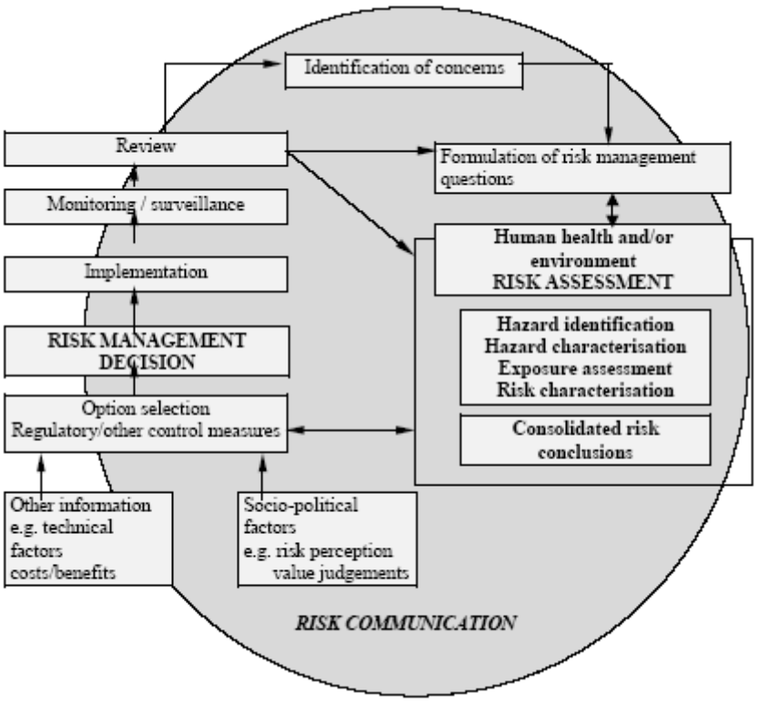

Figure 1-1 The "Risk Cycle" (European Commission, 2003b)

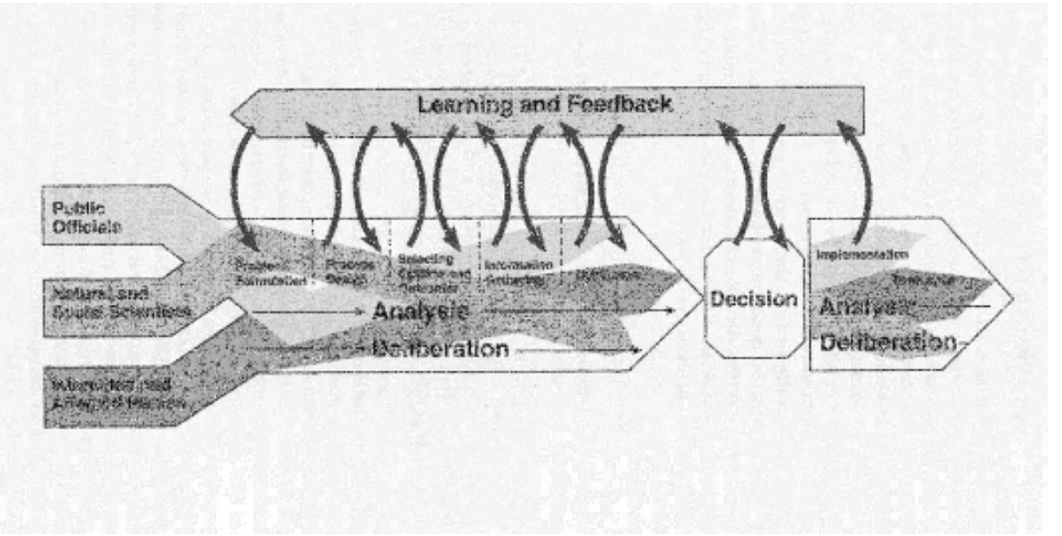

Figure 1-2 Understanding risk: informing decisions on risk in a democratic society (US National Research (US National Research Council; NRC, 1996) Reprinted with permission from the National Academy of Sciences, courtesy of the National Academies Press, Washington, D.C.

The ENTRANSFOOD project has addressed such concerns to some extent by inviting consumer groups and industry representatives to participate in the project; the basic concepts of risk assessment and risk management, as defined in current international and European policy documents, however, were maintained. The achievements and lessons from the attempt of addressing both scientific and societal aspects of the placing on the market of GM food crops in the ENTRANSFOOD project are further discussed in Chapter 7.

The international guidelines for risk assessment are largely based on the 1983 report of the National Research Council on risk assessment in the Federal US government; international expert groups under the auspices of Codex Alimentarius, the FAO, and the WHO further elaborated the concepts described in this report. Risk assessment involves gathering information on severity of the effect that a hazard might create and on the potential degree of exposure to hazardous substances. This originates from a concept already formulated in the middle ages by Paracelsus at the turn of the 16th Century, who stated "All substances are poisons, there is none which is not a poison; the right dose differentiates a poison and a remedy". Risk assessment is often grouped into four activities: hazard identification, hazard characterisation, exposure assessment, and risk characterisation. The first step in risk assessment is thus to identify a hazard, by establishing its potential to cause harm using toxicological experiments and assessing information on the relation of exposure and effect across whole populations.

Hazard characterisation aims to evaluate in qualitative and quantitative terms the nature of the identified hazard. This usually involves an analysis of dose-response relationship of harmful effects in a test animal or other test system and characterisation of the severity of the effect. In routine toxicological analysis, animals are administered usually three doses, including a dose that exceeds anticipated human exposures by several orders of magnitude. Observation and autopsy of animals helps to establish the highest dose levels at which no adverse effect occurs – the No Observed Adverse Effect Level (NOAEL).

Information on the quantity and distribution of a potentially hazardous substance in the environment is then required in order to determine where populations are expected to come into contact with the substance; for foods, dietary intake assessments of populations are required. Particular attention is paid to expected average and worst-case intake levels of the most sensitive subgroups of a population. This information is used to determine the population groups that may be at risk and the distribution of such risks. These are the elements that allow estimating the probability that harm will occur. Exposure assessment often needs to take into account important societal factors necessary to anticipate behaviour of a wide range of individuals that might affect their exposure.

Risk characterisation then combines information about the probable extent, nature, and duration of exposure with considerations of hazard characteristics and relevance of those hazards for humans into an integrated view of the likely risk to human health. Any uncertainties inherent in the risk assessment should be highlighted. This information then constitutes the basis for a determination of a dose that is deemed to be safe. If the expected intake exceeds that dose, risk mitigation measures, such as restrictions on use of a chemical, have to be adopted. Recently, the EU Concerted Action "Food Safety in Europe (FOSIE)" has reviewed risk assessment strategies and methods for chemicals in food and diet. Building on FOSIE's findings, one objective of ENTRANSFOOD was to assess the merits and limitations of using existing toxicological methods developed for the assessment of chemicals, such as food additives and pesticides, for the safety assessment of whole GM plant-derived foods.

1.3 Risk assessment strategies for whole foods

Health is a common association with plant-derived foods; such foods are known to be sources for beneficial dietary components, such as nutrients, minerals, and vitamins. However, some food crops and vegetables also contain toxic compounds: potatoes contain glycoalkaloids, and raw courgettes contain coumarins that, at high intake levels, are considered carcinogens. The regulator's awareness that traditional plant-derived foods are associated with both benefits and risks has been heightened by deliberations on how to assess the safety and wholesomeness of GM food crops.

Few plant-derived foods have been tested using toxicological methods. Most countries require measuring the levels of known natural toxicants for the registration of new traditionally bred crop varieties. Testing whole foods in laboratory animals is challenging. Two basic questions need to be addressed: Can we feed the test animal with doses that are sufficiently high to induce adverse effects? Can we compose a diet for the test animal that respects the nutritional needs of the animals? Whether animal tests can provide meaningful information towards the safety assessment of a plant-derived food depends on the type of food and the level and type of anti-nutritional compounds in food. The safety of whole plant-derived foods is usually based on a long-term experience and history of safe use, even though such foods may contain anti-nutritional or toxic substances. This concept is the starting point for the safety assessment of foods derived from GM crops.

1.4 Safety assessment strategies for foods derived from GM crops

Do risks associated with foods derived from traditionally bred crops and GM crops differ? Techniques for genetic modification allow the transfer of genetic material across species. Changing the genome can result in changes in the plant's development and metabolism. Safety assessment of GMOs therefore requires a detailed understanding of the transformation process, the introduced genes and gene products, and possible alterations in the composition of a modified plant (see Figure 1-3). Potential hazards to human health may result from changes of the content of toxicants, allergens, or nutrients; potential adverse consequences from the transfer of the recombinant DNA from modified plants to other organisms, such as microbes in the human gut, should also be considered (horizontal gene transfer is addressed in more detail in Chapter 4).

The challenge in assessing the safety of GM crops is to characterise the properties of new gene products and potential changes in levels of endogenous plant constituents, and to identify potential unintended (unexpected) effects due to the genetic modification that may have adverse impacts on human health or the environment. Changes in plant genomes due to unintended effects from genetic modifications can also occur in traditional breeding (see Chapter 3 for a more detailed discussion of this point). Experts under the auspices of the OECD and the United Nations' World Health Organisation (WHO) and Food and Agricultural Organisation (FAO) have developed approaches for the safety assessment of foods derived from GMOs. The European Commission also recently published a more detailed guidance document on data requirements for the safety assessment of GM crops.

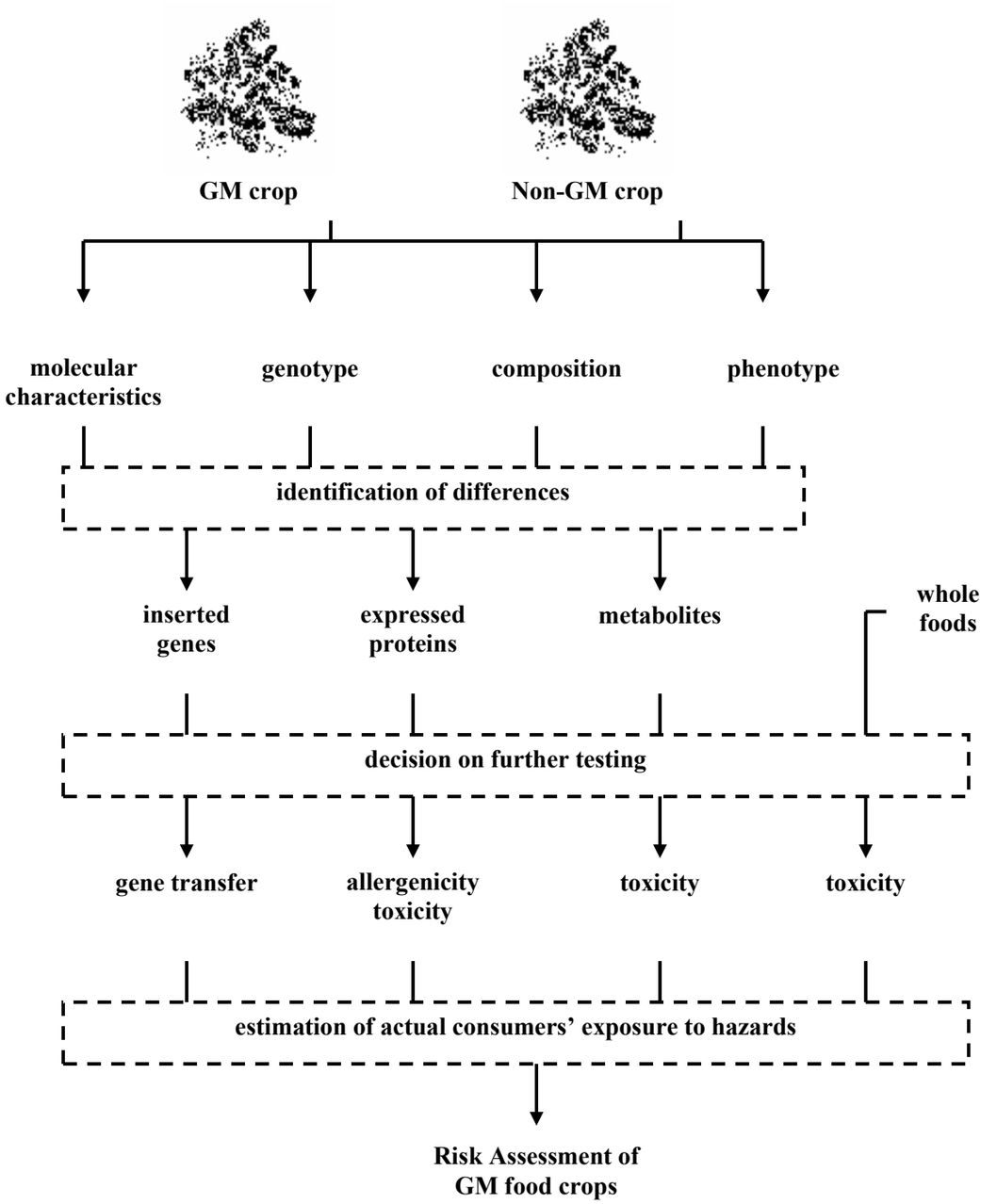

Figure 1-3 Risk assessment strategy for GM food crops (designed by G.A. Kleter)

The safety assessment of foods derived from GM crops relies on the comparison to foods derived from a close non-modified counterpart that has a long history of safe use. Most methods for the safety assessment of foods derived from GM crops are adapted from methods used to assess the safety of chemicals found in foods, such as pesticides and food additives. The application of this concept of *Substantial Equivalence* serves to identify potential differences between the GM food crop and its counterpart. Any identified changes are then the subject of assessment in order to determine their potential impact on human and animal health. The concept of substantial equivalence is thus a *guiding principle, or starting point* for the safety assessment of new foods, not an *endpoint* of a chemical comparison between the new and the traditional product (see Box 1-2).

BOX 1-2

SAFETY ASSESSMENT OF FOODS DERIVED FROM GM CROPS

Comparative safety assessment taking conventional crops as safety standard

Comparative analysis of the agronomic and compositional properties of the GM crop and its traditional counterpart

Safety and nutritional assessment of identified differences

A product-specific safety assessment is carried out in case of GM foods with no traditional counterpart

Critics of biotechnology pertain that current targeted testing approaches do not sufficiently address putative unintended and unexpected effects and cannot rule out occurrence of potential long-term effects that result from sustained human exposure to crops with potentially hazardous subtle changes in a plant's composition. The use of the concept of Substantial Equivalence has been seen as an excuse to avoid extended toxicological testing with animals. The same critics are concerned that a targeted risk assessment approach based on chosen parameters is insufficient, as it does not address the risk of activation of putative, previously silent and unknown genes for allergens or biosynthetic pathways of toxic secondary metabolites. Furthermore, critics complain that there is too little guidance for producers on what parameters should be measured for the comparison, by what methods such data should be obtained, and how samples should be obtained from which type and number of field trials in order to allow a statistically sound analysis of the data. Many of the criticisms of the Concept of Substantial Equivalence also are based on a misunderstanding that the concept is used as an endpoint and assessment outcome, rather than as a guide to further testing of identified changes. However, no alternative approaches to the concept of substantial equivalence for the safety assessment of GM food crops have been proposed.

The current approach for the safety assessment of GM crops that is based on the concept of substantial equivalence has been widely adopted in both the public and private sector; it is considered the best available approach. Guidelines to its implementation are becoming ever-more standardised and detailed. For example, the Organisation for Economic Cooperation and

Development (OECD) is compiling consensus documents for certain crop species that provide the information considered of most relevance for the characterisation of the parent crop. Two types of consensus documents are available for the world's major food crops: the first describes a crop's biology, focusing on attributes that are relevant to the environmental safety assessment, and the second describes a crop's compositional characteristics that are of most importance for the food safety assessment. All documents are on Internet (OECD, 2004ab). Guidelines on the application of the concept will become increasingly detailed and differentiated for different product categories.

Scientific concepts, including the concept of substantial equivalence, continue to develop driven by science and society. It is important to acknowledge that, like our understanding of science, our understanding of risk will always remain "incomplete", but that decisions on risks and benefits of new technologies should be taken regardless of knowledge waiting to be discovered. Scientists, regulators, politicians, and citizens alike should embrace this political dimension of our understanding and weighing of risks.

In conclusion, approaches to regulation and conditions for the approval of GM crops differ across jurisdictions. The same approach to the safety assessment is, however, adopted by regulatory authorities and public and private firms developing GM crops for commercial use in most countries. Continued improvement of methods for the assessment of transgenic crops will in future allow further reductions of some of the uncertainties associated with genetic modification. Dialogue between experts and civil society may contribute over time to further clarify and structure regulatory and risk analysis and strategies to improve the salience of assessments to address concerns of policy makers and the public.

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CHAPTER 2

SAFETY EVALUATION STRATEGIES FOR FOODS DERIVED FROM GM CROPS

Food safety is a relative concept. International guidelines on food safety declare a food safe when "there should be reasonable certainty that no harm will result from intended uses under the anticipated levels of consumption" (OECD). Since the first hunter-gatherers turned into settlers, continued improvement of food crops to enhance yields and facilitate cultivation has been central to development in all societies. All traditional food crops we consume every day have been steadily improved through selection and breeding over millennia; however, few have been subjected to a formal safety assessment. During the last two decades, more attention has been directed to food safety; novel foods and chemicals that enter the food chain are regulated and now have to undergo a testing regime. This chapter explains the strategy and the methods used to assess whether foods derived from GM crops are as safe and nutritious as other plant-derived foods that have become staples in our diet.

In this chapter, we present a systematic approach how to tailor the safety assessment of GM crops to the specific characteristics of the modified crop and the introduced trait. The approach is built on existing international guidelines for the food safety assessment of GM crops, provides, however, more detailed and explicit instructions on the selection of appropriate test methods (for a more elaborate description of this approach, please refer to König et al., 2003). First, we provide an overview on existing test methods developed for chemicals, including food additives, examining each method's suitability to test the safety of foods derived from GM crops. Second, we summarise how to determine whether the GM crop is 'as safe as' a suitable comparator with a history of human consumption. Traditionally bred crops serve as a safety standard: the safety of foods derived from GM crops is assessed through comparison to foods that have a history of safe use. Third, we outline the main implications of advances in molecular biology and the development of new *in vitro* and *in vivo* test methods for the future refinement of food safety assessment strategies.

2.1 Methods for toxicity testing

Regulatory requirements for chemicals such as food additives and pesticides, many of which were first instituted in the 1970s, have led to the development of a battery of tests to assess the safety of chemicals in foods. Strategies for assessing the food safety of chemicals usually combine three lines of evidence: investigation of the structure/function relationship for indications of potential toxicity and allergenicity; *in vitro* assays with enzymes, receptor proteins, or cultured cell lines; and *in vivo* animal studies.

Investigating the structure/function relationship. A test substance's physico-chemical properties, structure, and function can in some cases provide indications for potential adverse health effects. This information helps to frame the test approach. Some toxicants show a clear structure/function relationship and their mechanism of toxicity is fully understood; other classes of toxicants just share common structural elements or physico-chemical properties that may be indicative of toxicity. A molecule's physico-chemical properties help for instance the prediction of its propensity to intercalate in DNA and interfere with DNA replication; such interference can lead to mutations and cancer. Computer data bases help to assess whether a molecule shares characteristics of known toxicants: some data bases provide lists of toxicants and their properties; others use algorithms to predict a molecule's function through structural and physico-chemical characteristics.

In vitro methods. In vitro, or test tube methods were developed to test chemicals, including food additives, to serve as indicators for specific toxic effects. Some assays can help to assess whether molecules are stable under diverse conditions. Other assays assess whether molecules bind to, inhibit, or stimulate proteins with specific functions. Yet other assays test whether substances affect cell growth of diverse cell types. The methods can, in theory, serve either as screening systems to assess potential toxicity of a compound, or for investigations of a toxicological mechanism underlying a specific effect observed *in vivo* or predicted from the structure of a molecule. Very few *in vitro* tests are, however, validated formally, or used for safety tests submitted in regulatory applications. The interpretation of results from *in vitro* tests is challenging *in vivo* situations; uncertainties have to be clearly stated. The existence of potential harmful effects from ingestion of a substance should not be inferred from *in vitro* methods alone; such methods, in particular where they might indicate a potential adverse effect, should be used in conjunction with other test methods, including animal testing.

Animal models. Animal methods provide a holistic approach to safety assessment. A variety of standardised and validated laboratory animal tests have been designed to identify and characterise health hazards associated with exposure to single defined chemicals. In routine toxicological analysis, animals are administered usually three distinct doses of the test substance ranging from high to low. The high dose, where possible, exceeds anticipated human exposures by several orders of magnitude. The use of animal methods is, however, not without challenges; these challenges include the need to extrapolate from responses induced in animals to likely impacts on human health. These uncertainties are usually taken into account by adopting safety factors. Observation, clinical investigations, and autopsy of animals establish the highest dose levels at which no adverse effects occur: the No Observed Adverse Effect Level (NOAEL). The NOAEL is the basis for establishing best estimates of safe exposure levels or an Acceptable Daily Intake level for humans (ADI; Figure 2-1). For instance, in order to determine an ADI for humans from a rat study, the NOAEL is divided by an uncertainty factor, normally 100, in order to account for the following uncertainties: extrapolating test results from rodents to humans and differences in susceptibilities for toxic effects of the chemical between individuals in the human population. Animals can be used for the identification of acute toxicity, usually involving administration of a large single dose followed by 14 days of observation. Sub-acute, sub-chronic, and chronic toxicity or carcinogenicity is tested in animals over prolonged periods of one or several months, or the lifetime of an animal.

Animal tests for whole foods are much more challenging to design than tests for a discrete chemical or protein: difficulties include that animals (or man) cannot ingest multiples of the anticipated human consumption levels of the test substance due to its sheer volume. Furthermore, nutritional imbalances can arise if an animal's diet contains large proportions of a food it does not habitually eat. In laboratory tests, each animal species has its own specific dietary requirements of certain minerals and vitamins. Studies in which the test material is administered at the expected level of intake and at low multiples of that level, and in which the nutritional balance of the animal's diet is not disturbed, can provide safety assurance that the consumption of certain amounts of the new food will not induce adverse effects in animals.

Some of the methods developed for chemicals have been adapted for use in assessments of foods derived from GM crops. For example, an understanding of the structure-function relationship of newly introduced metabolites and proteins is the first step in any assessment of a GM crop. Databases exist for proteins with toxic or allergenic properties. The resistance of proteins to proteolytic digestion in the gastro-intestinal tract is measured by determination of stability in a simulated gastric fluid and sometimes also in a simulated intestinal fluid. Animal methods, if deemed necessary, can be adapted to test for potential adverse effects of recombinant proteins or

novel metabolites. Animal tests can, in some cases, also provide worthwhile information for the assessment of whole foods derived from GM crops. These methods are described in more detail below.

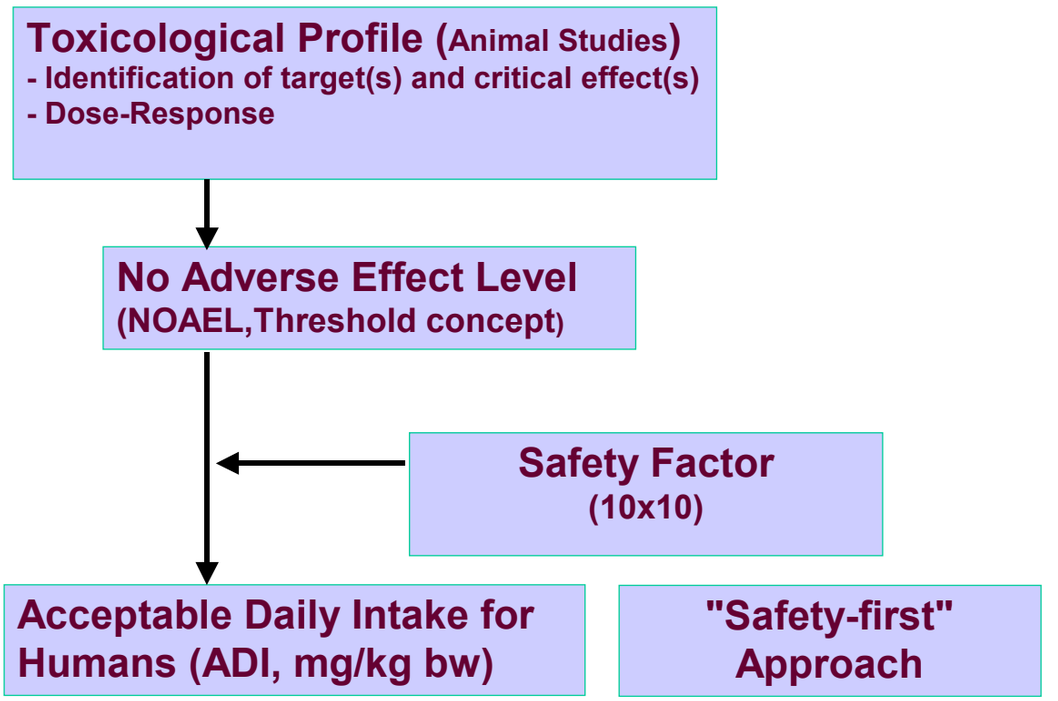

Figure 2-1 Safety evaluation of chemicals

2.2 Strategy for safety assessment of foods derived from GM crops

Safety considerations for foods derived from GM crops are fundamentally the same as those for conventional foods. The safety of whole plant-derived foods is a fundamental assumption based on a long-term experience and history of safe use, well knowing that such foods may contain anti-nutritional or toxic substances (see Section 1.3). This concept is the starting point for the safety assessment of foods derived from GM crops. The safety assessment therefore determines whether the GM crop is as safe as its conventional counterpart by identifying significant differences that occurred through the genetic modification that might potentially adversely affect human health. Furthermore, uncertainties associated with unintended changes in plant genomes through the insertion of recombinant DNA should always be considered in the light that crop genomes are constantly changing through a broad range of natural and man-mediated mechanisms. Subtle unanticipated changes in a plant's composition that may be difficult to detect using this approach can occur through genetic modification, traditional breeding methods, and natural genome rearranging processes. Therefore, studies with the whole food derived from GM crops may be carried out.

The safety assessment is conducted in four steps: the description of the parent crop; the description of the transformation process; the safety and allergenicity assessment of the gene

products and metabolites; and the combined safety and nutritional assessment of the whole plant (see also Chapter 1 and Figure 2-2). These steps are considered in turn.

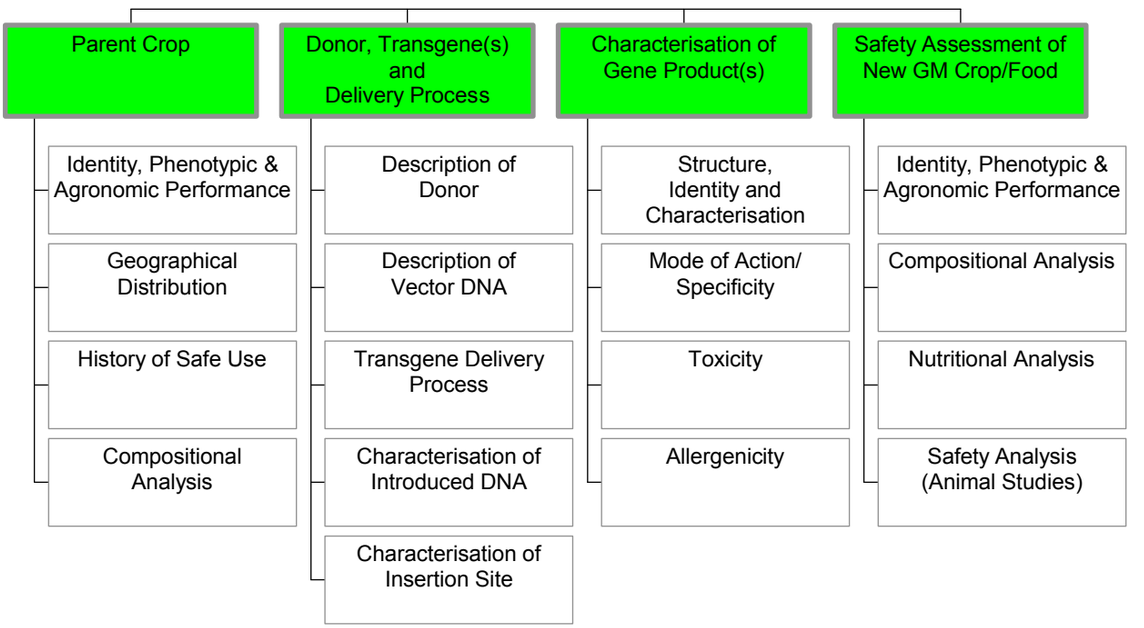

Figure 2-2 A fully integrated and iterative approach to the hazard assessment and characterisation of all elements involved in producing a new GM variety (König et al., 2004)

Description of the parent crop. The parent crop, and in some cases close relatives, should be characterised to understand whether the crop contains toxicants, allergens, or substances with pharmacological, or anti-nutrient effects. The characterisation of the parent crop then guides the choice of test parameters for the comparison of the GM crop to a close comparator, which is usually the non-modified parent crop. The OECD has developed consensus documents on the biology and compositional characteristics for the major crop species. These are intended to guide the description of the parent crop and the subsequent assessment of the GM crop.

Description of the gene donor, transgenes, and delivery process. Organisms from which the recombinant DNA has been derived, all transferred genetic elements, and the gene delivery process require a full description. The introduced DNA should be shown to be unrelated to any characteristics of the donor organisms that could be harmful to human health. A risk assessment of gene transfer to human cells or microbes should also be conducted where appropriate (see Chapter 4).

The provision of sequence information on the junction of the inserted recombinant DNA and the plant genome is required under European Community law to allow the development of transformation-event specific detection methods by regulatory authorities (see Chapter 5). Such sequences may help to characterise the insertion locus to predict if important plant endogenous

genes might have been disrupted through the genetic insertion event and ensure that no unintended fusion protein is produced at the junction of the plant and recombinant DNA.

Assessing recombinant proteins or metabolites. The first step in a hazard assessment is usually to understand the mechanism of action of the test substance. For example, recombinant proteins that confer resistance to insects derived from *Bacillus thuringiensis* are toxic to selected insects, but not to mammals. Binding to the insect gut triggers a conformational change, the protein then forms a pore through the gut cell membrane causing damage to the insect gut. Mechanistic studies have shown that these proteins bind to specific receptor proteins on the insect gut wall with differing selectivity and at alkaline pH, but do not bind to cell surfaces in the human gut.

The purified proteins and/or metabolites should be thoroughly investigated using classical approaches for defined chemical substances described for chemicals, provided such data do not already exist. Proteins that can confer desirable traits to crops are subjected to toxicological testing before a decision is taken on their use for development of a product for commercialisation. Any significant unexpected changes in levels of substance(s) detected during compositional analysis will require identification, characterisation, and safety assessment. If fusion proteins are expressed, these would need to undergo the same safety assessment as intentionally introduced recombinant proteins.

Knowledge of the amino acid sequence of the recombinant protein allows screening computer databases for any sequence similarities with known protein toxins and allergens. Limitations of the method include that not all structural properties that mediate allergenicity may be detected or that there can be false positive matches of a protein's structural element that is similar to an allergen's structure, but that does not mediate allergenic effects. A protein's physical stability and its stability in simulated digestive conditions are deemed to be indicative of potential induction of allergic responses and/or adverse health effects. The test is one indicator as to whether the recombinant protein shares the characteristic of stability to digestion under these conditions that is common to many allergens.

Repeated dose studies with recombinant proteins or derived substances are recommended to identify potential adverse long-term effects unless there is sufficient information to confirm the lack of toxicity or pharmacological activity of the recombinant proteins and metabolites, or unless there is extensive experience with these substances (for instance, from a history of safe use). It can, however, be difficult to obtain sufficient quantities of purified recombinant proteins for testing animals over prolonged periods of time. Protein levels in plant material are often too low to justify its use in animal tests. The assessment of protein toxicity therefore often requires purifying sufficient amounts of the heterologous protein from the GM crop or from other hosts (e.g. bacteria, yeasts) that have been genetically modified to over-express the protein for testing. Expression of genes in different organisms (plants or bacteria) can potentially result in differences in folding or post-translational modification of proteins; these need to be taken into account in the assessment. Typical parameters considered in demonstrating the equivalence between a protein that is produced in a plant and the same protein produced by bacteria include molecular weight, amino-acid sequence similarity, post-translational modification (e.g. level of glycosylation or phosphorylation), immuno-equivalence, and the activity and specificity of the reaction when the gene product is an enzyme.

The judgment on whether a protein is a likely allergen is based on a weight of evidence approach, and results of all tests must be taken into consideration, since no single test is sufficiently predictive. First, it is assessed whether the protein shares primary amino acid sequence similarity with known protein allergens. In addition, there appears to exist an association between the

ability of proteins to induce food allergy and the ability to resist digestion in the intestinal tract, and therefore the stability of proteins in simulated gastric fluids is considered as a useful criterion in the assessment of the potential allergenicity of GM foods. However, it is emphasised that novel proteins may exist that are stable for digestion and not be allergenic. Where the protein is derived from an allergenic source or where there is significant sequence similarity with a known allergen, assessments are made using serum from allergic patients to determine whether these exists serological identity with known allergenic proteins. This approach has allowed the identification of plant allergens in the past: For example, the development of soybeans with improved nutritional characteristics for feed use by transferring a protein from Brazil nut was terminated, when the allergenicity assessment revealed that the selected protein was allergenic; the product was therefore never developed for commercialisation. The Codex Ad Hoc Task Force on Foods Derived from Biotechnology suggests that further improvement of methods for targeted screening of novel proteins with serum of allergic patients and the development and validation of animal models could enhance in future this weight of the evidence approach; it also recommends the establishment of international serum banks for such purposes and further research into T-cell epitopes and other structural motifs associated with allergens.

Combined safety and nutritional assessment of the whole plant. The GM crop is compared with the parent crop in order to identify all major differences between them. Any significant differences in agronomic, physiological, and compositional characteristics between the GM crop and the conventional counterpart are then subject to further testing to assess potential health implications. Knowledge of the characteristics of the crop species that is transformed, the introduced recombinant material, and the source organism of the recombinant material guides the selection of the parameters for this comparison. Parameters can also be selected based on knowledge of the plant genome sequences at the recombinant DNA insertion site.

Selected compositional parameters are representative of the main metabolic pathways in the plant and reflect potential consequences from the introduced trait. The assessment focuses on those that might affect human health, such as key nutrients, anti-nutrients, and allergens. This *targeted* approach is deemed appropriate for the evaluation of "first generation" of GM crops with relatively simple genetic modifications that are largely aimed at improving specific agronomic characteristics. It is important that these comparative studies on the composition are carried out under well-defined conditions at different locations and identical for the parent and the modified crop. Statistical analysis of the results is required.

Animal tests with whole foods derived from GM crops are considered to contribute useful information in the safety assessment if the composition of a GM food crop is modified substantially, or if there are any uncertainties on the equivalence of its composition to a traditional counterpart. In these two cases, dietary sub-chronic rat studies are recommended to demonstrate the safety of the food. Sub-chronic dietary studies with rats serve as an indicator that there are no unintended changes in foods derived from GM crops that might render it less safe, or more hazardous to health, than the comparator. Carefully designed diets, selection of doses, and feeding protocols are required to address challenges in animal studies with whole foods described in Section 1.3. Any potential preliminary evidence on unexpected changes that potentially adversely effect human health, but which may not necessarily indicate that the new food is not suitable for consumption, requires further nutritional or toxicological investigation if such preliminary indications do not deter plans for the product’s commercialisation.

In order to complement classic toxicological studies in rodents, studies in young fast-growing animals such as broilers are sometimes used for investigating potential effects of whole foods on the growth rate of individuals. Ethical consideration should, however, also guide decisions on the

use of animals in testing and the design of test protocols. Many scientists consider that studies in fast-growing species should only be deemed necessary in cases where there is reason to believe that a plant's metabolism may be altered such that its nutritional or toxicological characteristics are changed (see also Box 2-1).

BOX 2-1

LIMITATIONS OF ANIMAL FEEDING STUDIES WITH WHOLE FOODS

Bulkiness of test material

Formulation of diets

Palatability

Limited dose-range

Small safety margins, if any

Confounding factors / interactions between food components

Nutritional imbalances

Insufficient sensitivity for specific endpoints

Exposure and safety assessment. Human exposure to foods derived form a particular GM crop can be estimated combining data on the consumption of the traditional food crop with estimates of the proportion of that crop that is genetically modified. Potential changes in intake pattern due to the new trait have to be taken into account.

2.3 Considerations for GM crops with altered nutritional properties

The safety testing strategy described is also applicable to new generations of GM food crops with extensive compositional changes. For GM crops that have been modified extensively such that there is no single crop that is a conventional counterpart suitable for comparison, all new substances or existing substances whose levels have been altered should be assessed on a case-by-case basis; safety studies with the whole crop should also be conducted.

The safety assessment of GM crops that are intentionally designed to be compositionally different requires increased attention to two issues: the choice of an appropriate comparator and the estimate of the anticipated exposure. One example of a compositionally altered GM crop currently under regulatory review is oilseed rape that contains lauric acid, a fatty acid not normally found at elevated levels in oilseed rape oil. The product was developed as a substitute for tropical oils (for instance, palm oil) in certain food applications. The comparator with safe use in this case was palm oil.

2.4 Post-market monitoring

Past attempts to investigate the correlation of incidence of adverse health outcomes and food intake by post-market monitoring of foods have proven difficult. In an OECD survey, responding government authorities indicated practical difficulties monitoring health effects associated with food consumption; challenges included defining the exposure groups and levels of exposure, as well as establishing a cause-effect link between eating a certain food or food ingredient and the manifestation of a particular health effect. Interactions between various food components with beneficial and adverse effects are usually too complex to allow proof of associations of a specific health endpoint with an individual food component. Furthermore, the definition of individual health effects to be monitored is difficult.

To date, no foods derived from GM crops have been placed on the market for which post-market monitoring was deemed necessary. The British Food Standards Agency (FSA) has commissioned a feasibility study to determine whether long-term monitoring of novel foods is possible. This study assesses the government's ability to detect variations of food purchasing and consumption at the district level in Great Britain, as this is seen as an indicator for the feasibility to detect and to link such variations to health outcomes.

The success of a post-market monitoring regime to assess health effects of foods derived from GM crops largely depends on whether specific health endpoints for monitoring can be identified, and on the marketing strategy of the particular food. A cause-effect hypothesis must exist, the testing of which is the objective of the post-market monitoring program. Health effects suitable for monitoring have clear symptoms with strong manifestations that occur shortly after food intake (such as an allergic reaction). Conversely, monitoring for longer-term or weaker effects is challenging, if not impossible. In the US, for example, it was attempted to monitor blood levels of vitamins and carotenoids in olestra consumers, as clinical trials demonstrated that olestra consumption reduces the absorption of such fat-soluble nutrients. Subjects ate less than expected; conclusions of the study are therefore only tentative: no effects were observed.

The marketing strategy of products also matters for the success of identifying exposed populations groups for post-market monitoring: consumption of branded products in which the product was effectively the sole route of intake of the ingredient of interest can be monitored successfully. Estimating intakes of the same food component from different sources may be difficult, as each company can only monitor its own products. Post-market monitoring of health implications of certain commodity crops used in a wide variety of food products that are consumed in parallel is likely impossible (see Box 2-2).

Post-market monitoring of foods derived from GM crops is therefore not recommended as a routine practice. It is expensive, sequesters scarce resources for studies of health and food, and is unlikely to provide meaningful information. Post-market monitoring might be considered for identity-preserved GM crops with changed nutritional characteristics in order to confirm the pre-market assessment: a clear test hypothesis in form of a causal relationship of food intake and health impact must be formulated.

BOX 2-2

FEASIBILITY OF POST-MARKET MONITORING

Post-market monitoring may be considered to:

- Confirm the pre-market safety assessment
- Identify unexpected adverse effects which remained unnoticed during the pre-market safety assessment.

Can health endpoints for monitoring be identified?

Does a cause-effect hypothesis exist?

Can consumption of the branded GM product/ingredient via a sole route of intake be monitored?

Intake of the GM foods via different sources is difficult to estimate

Health implications of commodity GM foods/ingredients are difficult if not impossible to assess.

2.5 Developments in food safety research

Advances in molecular biology, biochemistry, and nutrition will over time facilitate the development of new crop varieties. Applying new insights to refining and adapting safety assessment approaches to advances in product developments will be important. Recommendations on priorities for research and development of test methods and strategies are provided below, considering advances in molecular biology, allergenicity assessment, and safety and nutritional testing in turn (see also Box 2-3).

Molecular biology. Genomic research adds a new dimension to our understanding of biology and provides powerful new tools to study induced changes in gene expression. Our improved understanding of the structure of plant genomes, functions of individual genes, and a plant's responses to its environment at the molecular level will improve our understanding of the characteristics of the parent crop that pertain to food safety assessment. Results from large scale sequencing projects are rapidly increasing our understanding of plant genomes and of their evolution, regulation, and plasticity. The recent completion of the first draft sequences of the rice genome and the availability of the *Arabidopsis* sequence information now allow whole genome comparisons between different types of plants. More than 80% of the genes that were annotated in *Arabidopis* were also found in rice. Evolutionary biology and reverse genetics provide important information about the functions of individual genes. Improved understanding of plant genomes will reduce uncertainties of consequences of single insertions of recombinant DNA in a plant genome. The establishment of international systems for improved access to crop genome databases and latest bio-informatics methods in order to facilitate and harmonise the future analysis of such data is key. Our enhanced understanding of food crops and implications of consumption of diverse plant-derived foods on human health will, in the long term, also reduce uncertainties in food safety assessment.

BOX 2-3

FUTURE TARGETS FOR FOOD SAFETY RESEARCH

Development of micro-array systems for gene expression studies

Analysis of structure-function relationships of proteins that have the potential to cause allergic sensitisation

Development and validation of animal models for the prediction of allergenicity of proteins

Application of micro-array and proteomic technologies for the identification of new biomarkers for health effects of bioactive compounds in foods

Integration of *in vivo* and *in vitro* systems of animal and human origin for food safety testing

The availability of sequence information of entire plant genomes also allows the development of micro-array systems to assay induced changes in gene expression patterns. This will, in future, also allow assessing potential changes in gene expression in genomic regions that are adjacent to the insertion locus. The interpretation of such data will, however, be challenging, as a greater understanding of gene functions and changes in expression levels is required before the safety implications of any such change in gene expression can be assessed (see Chapter 3 for a more detailed discussion).

Future targets in allergenicity testing. In future, an increasing number of GM crops may be developed with proteins to which humans have not as yet been exposed; this highlights the need for further refinement of tools for assessing the allergenic potential of novel proteins. A more detailed appreciation of the ways in which protein structure can impact on allergenic activity will facilitate the development of robust methods for identifying and characterising proteins with the potential to cause allergic sensitisation. There is also a requirement for biochemical and immunobiological research investigating how protein digestibility influences the sensitising potential of proteins.

There is a growing consensus that the safety assessment of certain novel proteins to which there was no documented human exposure will require the use of appropriate validated animal models for characterisation of the allergenic potential. Several models have been proposed and some of these show promise. However, none has yet been fully evaluated or validated. The requirement is for the most promising animal models for allergenicity to be evaluated fully with a range of sensitising and non-sensitising proteins so that their sensitivity and selectivity can be assessed. At present, animal models for predicting and characterising protein allergenicity are based upon assessment of induced antibody responses. However, it should be possible soon to consider alternative or supplementary endpoints based on a more detailed understanding of the immunobiological basis for sensitisation and an appreciation of why proteins differ in their

sensitising potential. Research in this area will be facilitated by the availability of micro-array and proteomic technologies that should aid in the definition of appropriate markers.

Safety and nutritional assessment of foods. Advances in genomics and developments of toxicological methods will improve our understanding of health impacts of exposure to various substances in the longer term. The use of such methods as part of routine risk assessment strategy is, however, still some way in the future, as deduction of health impacts in populations is fraught with uncertainties and as such methods will require validation. For example, the development of genomic expression profiling using micro-array systems prompts research into biomarkers that will allow to assay and compare changes in gene expression upon exposure to specific toxicants and nutrients in different test systems: in cultured cell lines, animal models, and, where appropriate, humans. However, the extrapolation from changes in gene expression to manifestations of disease symptoms is challenging. The merits and limitations of profiling methods are described in detail in Chapter 3 that discusses their use for characterising unintended effects of the genetic modification. If reliable protocols for DNA micro-array systems that allow the detection of such subtle changes can be developed and validated, the comparison of *in vitro* responses of cell lines and animal models using such methods may help towards a more detailed understanding of some aspects of similarities and differences between *in vitro* and *in vivo* test methods. By comparing *in vitro* test systems with the same cell lines derived from different species or comparing *in vivo* test results in different species, such methods will also provide information on inter-species and inter-individual variations of responses.

One research project under ENTRANSFOOD investigates strategies for food safety assessment relying on the combination of novel diverse, but not as yet validated test methods: the SAFOTEST project investigates GM rice containing lectins, well-known toxicants in plants. Rat feeding trials are performed with diets containing parent rice, GM rice, or GM rice spiked with the lectin at a relatively high dose level that is known to be toxic. These experiments are paralleled by *in vitro* experiments on the digestibility and cytotoxicity of the recombinant proteins in intestinal epithelial cell lines derived from humans and rats. Changes in gene expression profiles in rat and human intestinal epithelial cell lines upon exposure to sub-cytotoxic concentrations of the lectins and their peptic-tryptic digests are determined using DNA micro-arrays. These *in vitro* profiles are then compared with the expression profiles in intestinal samples taken from live rats exposed to the same proteins during feeding experiments. This allows the comparison of results obtained from *in vivo* and *in vitro* systems of animal and human origin; if correlations are observed, both tests together have a greater (but still limited) predictive power than just one of them.

Finally, advances in information management will contribute to facilitating the safety assessment of GM crops. The establishment of international databases with information on the genome and the chemical composition of specific crops, as well as nutritional and toxicological tests conducted with both crops and individual compounds, including nutrients and toxicants, would immensely facilitate the case-by-case assessment of individual GM crops, in particular if such databases are linked such that they can be searched at the same time.

2.6 Conclusions

Safety considerations for foods derived from GM crops are fundamentally the same as those for conventional foods. The safety of widely consumed whole plant-derived foods is a fundamental assumption to this safety assessment approach, which is based on a long-term experience and history of safe use, well knowing that such foods may contain anti-nutritional or toxic substances. Uncertainties in this assessment associated with unintended changes in plant genomes through the

insertion of recombinant DNA should always be considered in the light that crop genomes are constantly changing through a broad range of natural and man-mediated mechanisms.

We developed a systematic approach how to tailor the safety assessment of GM crops to the specific characteristics of the modified crop and the introduced trait. The cornerstone of the safety assessment of novel foods, including foods derived from GM crops, is the concept of substantial equivalence. This concept prescribes the comparison of the GM crop to a suitable comparator with a long history of use that allows for identification of any significant differences that might impact human health. These differences then become the focus of further analytical, toxicological, or nutritional analyses. The safety assessment of foods derived from GM crops is divided into four steps: characterisation of the parent crop; characterisation of the transformation process; toxicological evaluation of new gene product(s) and allergenicity assessment; and nutritional and toxicological evaluation of the GM crop/derived food.

We recommend to conduct repeated dose studies with recombinant proteins or derived substances to identify potential adverse long-term effects unless there is sufficient information to confirm the lack of toxicity or pharmacological activity of the recombinant proteins and metabolites, or if there is extensive experience with these substances (for instance, from a history of safe use).

Current strategies for the assessment of potential allergenicity of recombinant proteins, as developed by the Codex Alimentarius Commission, are adequate. The judgement on whether a protein is a likely allergen is based on a case-by-case and weight of evidence approach. Methods for the assessment of the sensitisation potential of proteins need to be improved to allow the transfer of proteins that might share certain structural characteristics with allergens.

A more detailed understanding of protein allergy will enhance further safety assessment of a protein's potential for allergic sensitisation. We consider of particular value the development of an animal model that would permit the identification and characterisation of potential food allergens. Progress in this area will be facilitated by a more thorough appreciation of the factors that confer to proteins the potential to induce allergy and what distinguishes these from non-allergic proteins. Further research on the structure-function relationship of allergens is encouraged.

Animal tests with whole foods derived from GM crops are considered to contribute with useful information to the safety assessment. We recommend such tests should only be a requirement in cases where either the composition of the GM food crop differs significantly from that of its non-modified counterpart or the safety assessment approach provided any other indications for significant changes through the genetic modification that may potentially have adverse health impacts. In this case, dietary sub-chronic rat studies are recommended to demonstrate the safety of the food. Further standardisation of test protocols for animal feeding trials is recommended in terms of design of the diet, when, how, and how often the diet is administered.

ENTRANSFOOD does not recommend post-market monitoring of foods derived from GM crops as a routine practice. It is expensive, sequesters scarce resources for studies of health and food, and is unlikely to provide meaningful information. Post-market monitoring might be considered for identity-preserved GM crops with changed nutritional characteristics in order to confirm the association with a specific health effect: a clear test hypothesis in the form of a causal relationship between food intake and health impact must be formulated.

Genomic research adds a new dimension to our understanding of biology and provides powerful new tools to study induced changes in gene expression. Our improved understanding of the

structure of plant genomes, functions of individual genes, and a plant's responses to its environment at the molecular level will improve our understanding of the characteristics of the parent crop that pertain to food safety assessment. The establishment of international systems for improved access to crop genome databases and latest bio-informatics methods in order to facilitate and harmonise the future analysis of such data is key. Our enhanced understanding of food crops and implications of consumption of diverse plant-derived foods on human health will in the long-term also reduce uncertainties in food safety assessment.

In summary, it can be argued that the current safety assessment approach allows for the determination whether foods derived from GM crops are as safe as their conventionally bred counterparts; in some cases, GM crops are even better characterised than other non-regulated plant-derived foods. In conclusion, the current regulatory requirements and testing regimes are much more rigorous for GM crops than for conventionally bred crops. The safety testing strategy described here is also applicable to new generations of GM food crops with extensive compositional changes.

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CHAPTER 3

IDENTIFICATION AND ASSESSMENT OF UNINTENDED EFFECTS

Unintended effects are commonplace in attempts to improve plant varieties through both genetic modification and conventional breeding methods. Safety implications of unintended changes in plant-derived foods are difficult to assess, as plant foods are complex mixtures of constituents, many of which are bioactive. Few plant-derived foods have been subjected to safety testing. Science knows little about health implications of most minor constituents of plant foods. Furthermore, the levels of most constituents in plants vary depending on cultivation conditions and crop variety; for most plant constituents, the extent of natural variation is not as yet well understood. We define unintended effects resulting from genetic modification as "any significant difference in composition, morphology, or physiology between the new improved crop variety and the parent crop that is not related to the intentionally introduced trait". Evidence for significant unintended changes in crops is the trigger for further investigation in order to assess whether the changes may have any health implications.

In this, chapter we compare the origin and uncertainties in the assessment of unintended effects from genetic modification with those from traditional breeding. We also provide an overview on current and proposed future methods to identify and assess safety implications of unintended effects.

3.1 Sources of uncertainty about changed crop composition

Unintended effects from attempts to improve crop varieties may occur due to changes in the plant's genome or its protein content: the plant genome may be disrupted or changed at the site of insertion of recombinant DNA in the process of genetic modification, or from genomic rearrangements that occur frequently in the breeding process. Unexpected interactions can, however, also occur between introduced proteins (be it novel recombinant proteins or proteins from a related variety or species) and the recipient plant's metabolism, developmental, or physiological processes.

Unintended effects on the composition of a crop are of significance in a risk assessment if they adversely affect human health. Changes that have the potential to affect human health include increased levels of existing plant endogenous toxins or allergens; activation of the expression of previously silent genes encoding allergens or biosynthetic pathways for toxins; and lower levels of a nutrient essential in the human diet. Genetic changes in gene-rich and active regions of chromosomes are therefore more likely to have safety implications than changes in other regions. Natural recombination and genetic changes through plant breeding or genetic engineering are, however, more likely to occur in active regions of chromosomes than in inactive regions.

Natural plant genetic recombination mechanisms can be grouped into the two major mechanisms: (i) homologous recombination and (ii) illegitimate recombination. Recombination often occurs as a consequence of a double-stranded DNA breaks that occur during DNA replication or cell division. The cell's DNA repair proteins either fuse two DNA segments that have high sequence similarity (homologous recombination) or, occasionally and at a lower frequency, join the ends of two non-similar DNA strands (one form of this process is illegitimate recombination). Errors in these DNA repair processes that change the original sequence occur at relatively high frequency. The frequency of recombination varies across species. Some plant species, such as maize, contain transposons, selfish DNA elements that copy themselves and insert such copies spontaneously into new chromosome locations. Active transposons in plants can stimulate

homologous and illegitimate recombination in the vicinity of transposon-insertion sites. Transposon-induced recombination between direct repeated sequences and between sequences on different chromosomes has been reported. It has also been estimated that such transposon-induced recombination events between different chromosome segments could occur at frequencies of 10^{-3} to 10^{-5} per cell division. Induced DNA double-strand breaks can be repaired through recombination with DNA sequences from other chromosome segments. For a specific transposon in maize, it has been estimated that this process could result in chromosome inversions, deletions, or translocations 0.1% to 10% of the time.

Plant breeding relies on systematically identifying beneficial changes in crops resulting from natural genetic variation and on selecting these improved varieties for further propagation and finally cultivation. Breeders have also used artificial means to enhance genetic variation; these include the use of chemical or gamma irradiation mutagenesis to increase the error rate in DNA replication processes in plant breeding. The extent to which DNA structure and integrity are modified in these accepted breeding approaches is unknown. What is clear is that the genetic structure of plant populations has been widely changed by breeding practices, indicating the deep influence of breeding practices on the genetic make-up of plant crops.

Genetic engineering of crop plants largely relies on two methods used to introduce foreign DNA into plant cells: biolistic (microprojectile) bombardment, and *Agrobacterium*-mediated transformation. The biolistic method is based on a physical delivery of DNA-coated gold or tungsten microprojectiles into plant target tissue by acceleration. *Agrobacterium*-mediated transformation exploits the biological ability of this soil-borne bacterium to copy and transfer a specific portion of DNA (termed T-DNA) present on a tumour inducing (Ti) plasmid into the plant cell nucleus, where it can be integrated into chromosomes. Uncertainty on whether the genetic modification of a crop may result in unintended effects results largely from our lack of knowledge of the insertion location of the recombinant DNA in the plant genome. Unintended effects from the genetic modification may, for example, result if the insertion of recombinant DNA in the plant genome disrupts a plant gene, produces a fusion protein of recombinant and plant endogenous DNA, or alters gene expression levels in adjacent chromosome regions.

The process of transgene integration is identical to the preferred recombination mechanism that occurs in plant cells, especially during mitosis. Transgene integration in plants occurs through the natural ability of the plant's DNA repair system during DNA replication and cell division to join the ends of non-homologous DNA sequences. It is the same error-prone process that also is responsible for introducing several types of natural recombination events during the repair of double strand breaks in DNA. Gene disruptions, the production of novel fusion proteins, or changes in expression levels of plant genes may, however, also occur through natural genome rearranging processes (e.g. transposition as occurs in maize) and the use of traditional breeding methods.

Since transgene integration occurs in plants through illegitimate recombination mechanisms, in which no homology is required, it is not surprising to find that there is no preference for specific sequences in the genome for the integration process. At present, it is not possible to predict, from its nucleotide sequence, the fate and the site of the integration of a particular transgene construct in the plant genome. However, transgenes, in particular those containing T-DNA, do have preferences for gene-rich regions. Similarly, in traditional breeding, DNA recombination is frequent in gene-rich regions, thus giving rise to allelic variants that can be selected for if the effects are beneficial. Thus actively transcribed genes *per se* are hot spots for recombination in plants, regardless of whether the recombination event is inconsequential, leads to unintended

effects, benefits the breeding process, or serves to insert a recombinant DNA sequence during the process of genetic modification.

3.2 Identifying and assessing risks from unintended effects

Potential unintended effects from the genetic modification may be more easily detected than those from natural recombination events or mutagenesis events induced by man. For the former, the tools of molecular biology can be used to identify recombination sites based on the knowledge of the inserted DNA sequence, for the latter there is no knowledge on the DNA sequence at the recombination site. On the other hand, knowledge on epigenetic effects is still in its infant phase.

Plant breeding is an iterative process requiring experience with diversity in available genetic resources and familiarity with the variability within the species. In the early stages of conventional breeding, "low grade" selection processes are normally used to discard those plants that show visible changes that appear undesirable. Selection according to the optimal "visible phenotype" and screening for disease resistance can be included, for example, at an early stage. As the selection process continues, the sophistication of the parameters measured increases (facilitated by a reduction in the number of progenies) to encompass yield and quality traits. Extensive backcrossing procedures are applied in order to remove undesired unintended effects. Selection of the starting parental material is paramount in the breeding process. Due to the common practice of selecting favourable lines and discarding those exhibiting unwanted properties, unintended effects in conventional breeding are not frequently reported. The extent to which unintended effects occur during the course of a traditional breeding programme is almost impossible to assess. Unintended effects routinely occur and are propagated, some of which may have safety implications: for instance, potato cultivars were withdrawn due to unacceptably increased levels of neurotoxic glycoalkaloids, toxins present at low levels in all potatoes.

In GM crops, the knowledge of the introduced recombinant DNA sequence allows the identification of the insertion site in the plant genome. Sequence information obtained from the insertion site that bridges the recombinant DNA and the plant genome allows identification of potential fusions of recombinant and endogenous plant genes. More importantly, sequence information often also helps to identify whether important plant genes were disrupted or otherwise affected through the introduced recombinant sequence. It is then possible to formulate hypotheses on what might have been inadvertently changed in the GM crop. Such approaches could indicate if the insertion event occurred, for example, within or close to an endogenous gene or regulatory sequence. Our expanding knowledge of plant genomes and functions of individual genes, as well as the availability of associated bio-informatics and databases will increasingly help to reduce uncertainties on unintended effects from genetic modification.

The safety assessment of GM crops required for marketing aims to identify all major changes in the GM crop compared to the parent crop that might affect human health (see also Chapter 2). Any significant differences in agronomic, physiological, and compositional characteristics between the GM crop and the conventional counterpart are then subject to further testing to assess potential implications. The comparison is *targeted* according to predetermined criteria. The selection of the parameters is guided by knowledge of the characteristics of the crop species that is transformed, the introduced recombinant material, and the source organism of the recombinant material. Parameters can also be selected based on knowledge of the plant genome sequences at the recombinant DNA insertion site. Selected compositional parameters are representative of the main metabolic pathways in the plant and reflect potential consequences of the introduced trait; the assessment focuses on those that might affect human health, such as key nutrients, anti-

nutrients, and allergens. Moreover, all GM crops that enter the market have also undergone classical breeding and other processes for improving plant varieties.

BOX 3-1

STRATEGIES FOR DETECTION OF UNINTENDED EFFECTS

Targeted analysis	Non-targeted profiling analysis
Macro-, micro-nutrients	DNA analysis
Antinutrients, toxins	DNA / mRNA hybridisation
Specific secondary metabolites	Proteomics
	Metabolomics

3.3 Developments in detection of unintended effects

The development of complementary approaches to the targeted safety assessment approach is also viewed as particularly valuable for enhancing our understanding of plant biology in general and facilitating the development and the assessment of GM crops with more complex modifications. Complex traits, such as nutritional enhancements or tolerance to abiotic stresses, can fundamentally change a plant's metabolism and physiological processes. The more complex the modifications of the crop's composition, metabolism, or physiology, the more likely they are associated with unanticipated consequences. One R&D project that is part of the ENTRANSFOOD cluster, the GMOCARE project, is exploring whether profiling methods might contribute to the detection of compositional differences between GM crops and parent crops that might not be detected using the targeted comparative approach.

In order to increase the chances of detecting unintended effects, profiling methods have been suggested as tools to characterise changes in the composition of GM plants. The *non-targeted* approaches using DNA/RNA micro-array technology, proteomics, and metabolomics allow less biased analysis of possible changes in the physiology and metabolism of the modified host organism (Figures 3-1 and 3-2; Box 3-1). Advances in the development of the three types of profiling methods for plants are rapid, but due to difficulties of data interpretation, the added value of use of profiling methods in food safety assessment still remains to be proven. Potential merits and limitations of DNA and RNA micro-array technology, proteomics, and metabolite profiling are described in more detail below.

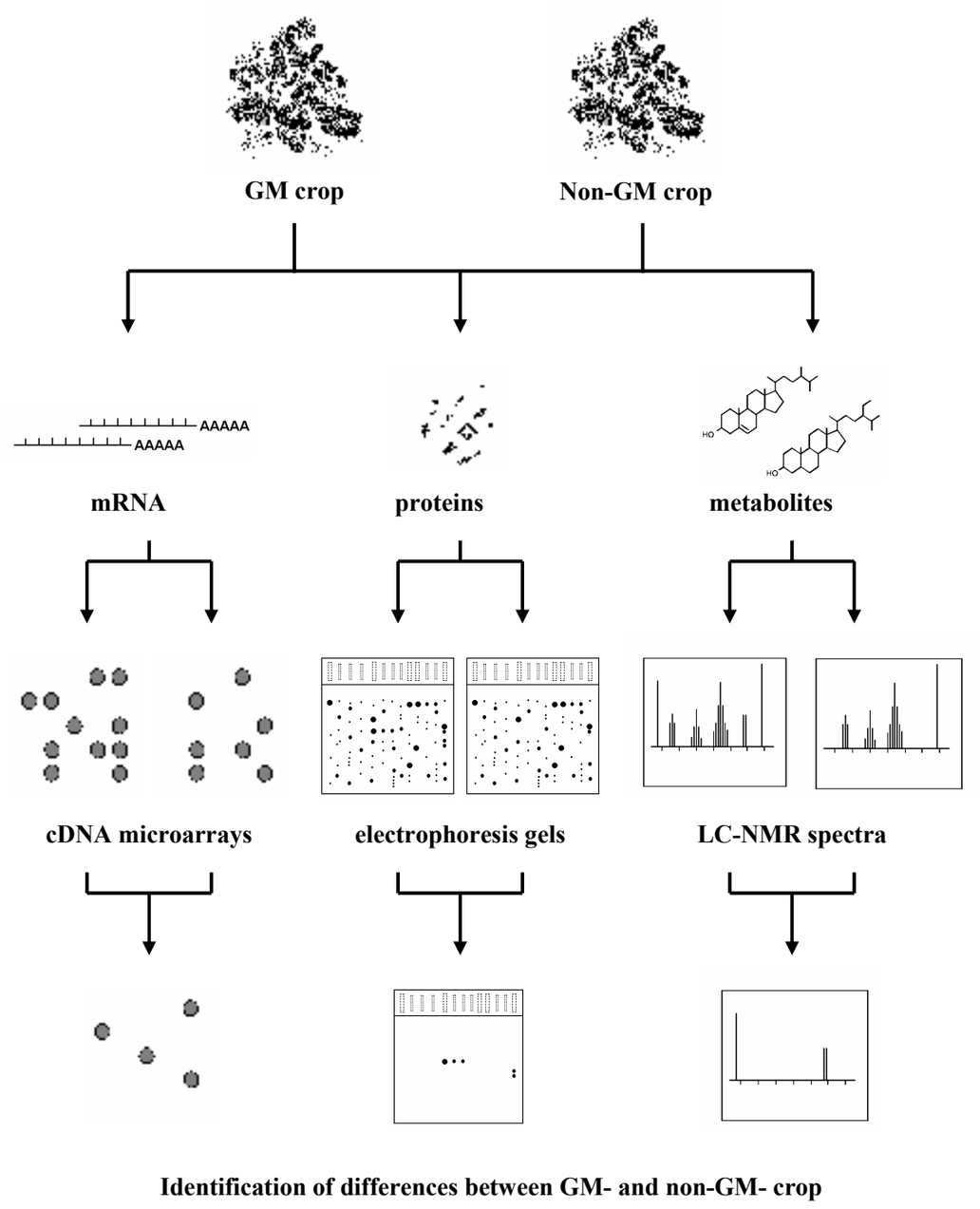

Figure 3-1 Profiling techniques for detection of unintended effects in food crops (designed by G.A. Kleter)

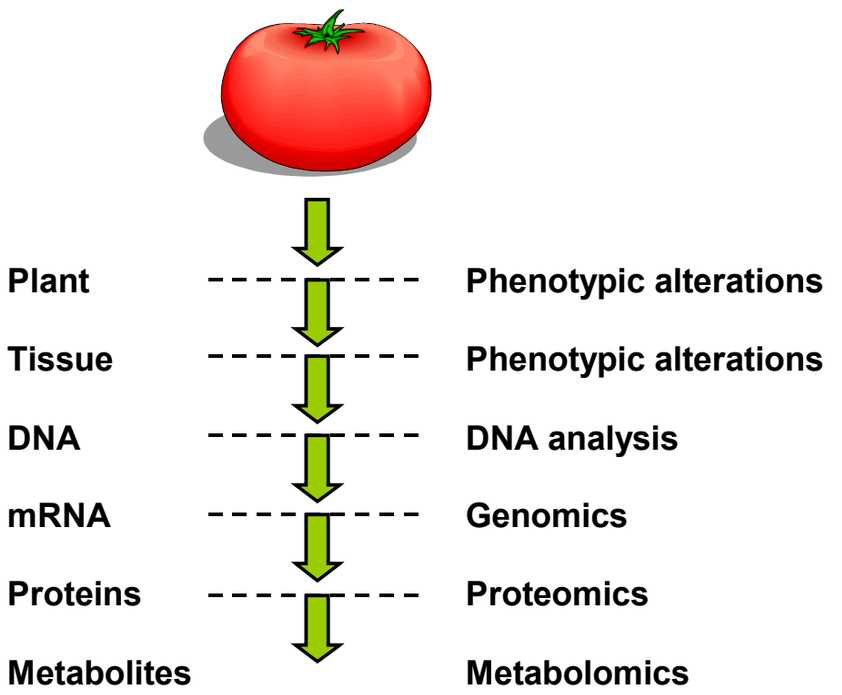

Figure 3-2 Integrated analysis of (un)intended effects in foods derived from GM crops (adapted from Kuiper et al., 2001)

Detection of altered gene expression. Development of DNA and RNA micro-array technology is an important step forward in the history of analysis of gene expression, facilitating parallel gene expression studies on thousands of different genes. Micro-array technology is based on hybridisation of mRNA to a high-density array (which consists of thousands of genes on an area no larger than a microscope slide) of immobilised target sequences, each corresponding to a specific gene. The technology is being tested for application in many fields, including medical, agricultural, and environmental science.

In order to study changes in patterns of gene expression in food plants, micro-arrays for the tomato and potato have been developed as model systems. Genetic modification may affect key metabolic pathways involved in the production of natural toxins and/or health beneficial compounds. Micro-arrays (and similar parallel gene expression technologies) allow simultaneous expression analysis of large numbers of genes thereby facilitating a broad-scale comparison of gene expression in the modified organism and its appropriate comparator. Where differences in gene expression occur, the availability of the DNA sequences of the genes in question will provide a first lead for further investigations into putative health effects from the observed differences. Observed differences in gene expression (i.e. mRNA levels) do not necessarily reflect parallel changes in the levels of the proteins the genes encode. The assessment of whether

there are health implications associated with the identified differences would therefore be challenging.

The construction of cDNA libraries representing different developmental stages of modified and non-modified organisms and subsequent hybridisation may yield important information on possible alterations in gene expression patterns. In this way, sufficient insight may be obtained into the *natural variation* in gene expression during different stages of development of the tissues of interest and under different environmental conditions. In the case of tomato, a green-specific library was prepared with the objective of isolating (amongst other genes) cDNAs that are related to the formation of natural toxins, such as tomatin. In contrast, the library prepared from red (ripe) fruit contains cDNAs associated with the metabolism of nutritionally important compounds such as vitamins and flavonoids. Thus, by constructing micro-arrays that are enriched in genes involved in several important pathways, the inadvertent effects of genetic transformation on such pathways can be monitored. Similarly, a potato library has been constructed that aims to further elucidate the metabolic pathways that contribute to the synthesis of glycoalkaloids, which are natural toxins. The outcome of these experiments will determine the usefulness of the micro-array technology in the screening for unintended effects in GM varieties associated with the genetic modification itself. From preliminary results, it can be shown that at least *intended* effects in GM tomato varieties can be monitored effectively.

Proteomics. Proteomics, that is the study of large set of proteins present in a cell, organism, or tissue under defined conditions, is a well-established technique that complements transcript (gene expression) profiling. The main approach currently applied involves two-dimensional gel electrophoresis followed by excision of protein spots from the gel, digestion into fragments by specific proteases, analysis by mass spectrometry, and subsequent computer-assisted identification using databases. This type of "differential display" proteomics has been applied to follow changes in polypeptide profiles and post-translational modifications induced by environmental factors or genetic mutations. One of the major challenges is the quantification of proteins. The range over which protein levels can be quantified is narrow.

The applicability of proteomic techniques to identify unintended effects resulting from the genetic modification of crops is being studied within GMOCARE. Detection of differences related to the genetic modification may, however, prove difficult, taking into account the large number of proteins present in a plant and the need to sub-fractionate the protein pool to detect minor components. As with all profiling techniques, one challenge is the high level of background “noise”, that is non-specific signals; this noise can be largely attributed to non-representative sampling procedures and genotypic variability. In order to overcome such challenges, it has been suggested that for safety assessment, a more targeted approach is more appropriate, such as a combination of immuno-blotting procedures and protein-micro-arrays, focussing on proteins involved in important metabolic pathways.

Metabolite profiling. The main approaches used for metabolite profiling are based on gas chromatography, high performance liquid chromatography, mass spectrometry, nuclear magnetic resonance, or Fourier-transform (near) infrared spectroscopy. These techniques can be applied in both targeted and non-targeted ("unbiased") approaches. By combining the various analytical approaches, it is possible to distinguish more than one thousand metabolites in plant extracts, but it is not yet possible to identify the majority of the constituents. The development of extensive mass spectral databases to aid metabolite identity in plants is a major challenge. There are several examples where metabolite analysis has detected unintended effects on metabolism. This includes, for example, lowered glycoalkaloid levels in GM potatoes with modified sugar metabolism and increased α-lycopene content in specific GM tomato fruit.

Within GMOCARE, targeted analysis of a range of GM potato lines and appropriate controls has been completed. Some lines exhibited extreme phenotypes, whereas no consistent differences could be detected between the GM potatoes and wild-type controls in, for example, glycoalkaloid (toxin) content and profile, sugar balance, fatty acid profiles, isoprenoid content, vitamin C content, and trypsin inhibitor activity. Similarly, using "unbiased" nuclear magnetic resonance analysis only one GM potato line with the most extreme stunted phenotype could be distinguished from its control (through modified proline content). These lines are now being subjected to gas chromatography combined with mass spectrometry and liquid chromatography combined with mass spectrometry.

To summarise, profiling technologies clearly provide powerful tools to enhance our understanding of changes in crop metabolism. A particular strength of micro-arrays is that they can contain the entire genome (provided the genome has been sequenced, as is the case for rice and *Arabidopsis*. Proteomic and metabolic profiling approaches are less comprehensive: plants contain many thousands of proteins and metabolites, only a fraction of which can be detected/identified/quantified using existing approaches. The three methods yield complementary information.

Profiling techniques yield large quantities of data Use of profiling to complement current approaches to safety assessment holds promises and challenges. Several issues need to be addressed before profiling approaches can become a proven and useful tool in standard risk assessment procedures. The method's comprehensiveness promises to identify any difference between new and old varieties. However, the interpretation of outputs (data) remains a significant challenge. Much work remains to be done in the development and standardisation of sampling procedures and approaches for data collection and handling. Inter-laboratory "ring" testing and validation of these methods will also be required. In addition, a more comprehensive understanding of natural variation in, for example, the levels of metabolites in crops needs to be developed to allow any unintended changes in a GM crop plant to be properly "benchmarked". For the comparison of large profiling data sets obtained for two different crop varieties, multivariate techniques, such as principal component analysis or hierarchical cluster analysis, are frequently applied. Such multivariate methods are useful, but discrimination between intended effects and unintended effects at the metabolite level may not always be possible. Therefore, perhaps most importantly, approaches will be needed to interpret the biological relevance and toxicological significance of any observed differences. Interconnected databases containing information on gene transcript, protein, and metabolite profiles for specific crop species at different developmental stages and grown in diverse environmental conditions would be helpful.

If, in future, reliable interpretation of results from profiling studies becomes a reality, such approaches will be useful to complement the current targeted safety assessment approach. The identification of possible differences between traditionally used and novel crops that might have adverse effects on human health is the overall aim of both targeted and non-targeted approaches.

3.4 Conclusions

Unintended effects on the composition of crop plants from one generation to the next can occur through natural recombination, mutagenesis approaches used in plant breeding, and genetic modification. There is no inherent unique risk in the deployment of recombinant DNA techniques. Variety selection and selection of parameters for both GM crops and conventionally bred counterparts that involve the assessment of physiology, morphology, and performance provide sound indicators of unintended effects.

Uncertainty associated with food safety of GM crops does not seem to be greater than uncertainty associated with conventionally bred crops. If anything, given current regulatory requirements specific for GM crops, they are better characterised than the conventional counterparts we eat. The safety assessment relies on a targeted approach of parameters indicative of the overall plant metabolism and possible changes from genetic modification. Such targeted approaches have proven to be effective for identifying unintended changes that might have implications for human health or the environment.

The application of profiling techniques will contribute to improving our understanding of the metabolic and compositional variations of crop plants and the potential alterations in gene expression, protein composition, and associated metabolic consequences that may stem from different cultivation conditions, breeding practices, or genetic modification. However, profiling methods are not as yet suitable for use in formalised risk assessments before we have sufficient data to understand natural variation in different gene expression and compositional parameters of crop plants, and before we are able to assess swiftly whether any observed significant changes in gene expression or composition may impact human health.

We recommend the allocation of public sector research funds to develop our understanding of food crops, including analyses using profiling methods. The development of profiling methods and international databases on natural variation in gene expression, protein, and chemical composition of crops should be encouraged. These methods will contribute to improving our understanding of the foods we eat and their potential implications for human health. Such methods will also facilitate the development and safety assessment of GM crops with more complex traits, such as nutritional enhancement and tolerance to abiotic stresses. Each of these methods, once further developed, may well be used to in future for certain categories of GM crops to complement the current targeted approach for the safety assessment of new crop varieties.

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CHAPTER 4

THE RELEVANCE OF GENE TRANSFER TO THE SAFETY OF FOOD AND FEED DERIVED FROM GM PLANTS

What are the risks from the transfer of recombinant DNA from GM crops to microbes or human cells? Since the first public discussions on genetic engineering in the mid-1970s experts agree that here is no inherent risk in the transfer of recombinant DNA between organisms, as all DNA is compositionally the same and not inherently toxic at typical human consumption levels. Although gene transfer between species is common in nature and has been a driving force in evolution, the risks from horizontal gene transfer of recombinant DNA from GM crops to other organisms should be assessed with care. Consequences of any gene transfer, natural or mediated by man, entirely depend on the function of the transferred gene in the recipient cell. In some cases, uncertainties in the assessment of the risks of gene transfer of a specific fragment of recombinant DNA may need to be addressed with further research or by improving the design of GM crops before a crop can be approved. Like science, understanding of risk will never be complete. This is, however, no reason to refrain from making decisions on new technologies that may hold some risks, but also promise benefits.

Assessing risks of horizontal gene transfer of recombinant DNA in foods derived from GM crops requires estimating both the likelihood of transfer of recombinant DNA from GM crops to microbes or human cells and the impact of such a transfer event. The risk is judged to be significant only if the recipient cell acquires a new function that may, directly or indirectly, have adverse effects on human health and if the acquisition of this gene by this cell type is significantly increased through marketing of the GM crop that contains the gene. Risk assessment of gene transfer of recombinant DNA from GM crops has focussed predominantly on the possibility that genes conferring resistance to specific antibiotics in certain GM crops may be transferred to microbes. In this chapter we discuss three issues central to the risk analysis of potential gene transfer from foods derived from GM crops to microbes or human cells: the occurrence and consequences of gene transfer between species; the risk assessment of antibiotic resistance genes; and best practices in the design of GM crops to reduce risks and uncertainties relating to gene transfer.

4.1 Occurrence and consequences of gene transfer between species

Rapid progress in sequencing genomes of diverse micro-organisms highlights the importance of horizontal gene transfer in microbial evolution: the identification of foreign DNA in microbial genomes provides evidence for frequent uptake and integration of foreign DNA. Stable integration of foreign DNA in a cell's genome and inheritance to subsequent generations requires four events: the uptake of foreign DNA, its effective replication in the recipient cells, expression of a new trait that confers the selective advantage, and a mechanism to pass the acquired DNA on to progeny in cell divisions. The uptake of genes from the environment or the transfer of genes between microbes enables microbial populations to rapidly respond to environmental changes. Lasting environmental changes may lead to alterations in the genetic make-up of a population over just a few generations, as the genetically best-adapted individuals propagate most effectively.

Genome sequences from complex multi-cellular organisms such as plants and animals provide evidence that gene uptake from the environment has not much contributed to genetic diversity. Mutations and the combination of different sets of chromosomes from two individuals through sexual propagation play a more important role in generating genetic diversity in higher

organisms. The human genome sequence contained no plant-derived DNA, very few bacterially-derived sequences, and many viral sequences; the sequences suggest that the integration of a foreign sequence in the human genome such that it is also found in germ line cells and inherited by subsequent generations takes place on a time scale of once in a thousand years. The factors affecting the likelihood of gene transfer to microbes and human cells, as well as possible consequences of such transfer events are considered in more detail below.

Gene transfer to microbes

The ability of microbes to acquire new genetic material varies between and within species. It can depend on environmental factors, such as nutrient availability and temperature. There are three main mechanisms that are of most relevance in the assessment of risks from gene transfer to microbes: conjugation, transduction, and transformation.

Microbes can exchange DNA by conjugation. Conjugation relies on self-contained, independently replicated DNA elements, such as conjugative plasmids. Genes contained on such plasmids usually include genes allowing adaptation to sudden changes in environments, such as genes conferring resistance to heavy metals or antibiotics, and functional elements allowing their replication and transfer from one cell to another. Other classes of "selfish" DNA elements can replicate and integrate themselves into host genomes; they, however, lack the cell-to-cell transfer functions, such as in the case of simple transposons. The most commonly used antibiotic resistance marker gene *nptII*, for example, was derived from Transposon Tn5 isolated from an *Escherichia coli* of a student's gut. Transposons are often found on conjugative plasmids. There are numerous examples of inter-species and inter-genus transfer of DNA by conjugation in food and in the intestine. In the risk assessment of gene transfer from GM crops, conjugation is of relevance, as any genes that are contained on conjugative elements that are wide-spread in microbial populations are more likely transferred between microbes via conjugation than via transformation. Conjugation has been observed to occur in the intestine.

Viruses can also transfer DNA from one cell to another by transduction. When viruses replicate in cells, cellular DNA fragments are sometimes accidentally packaged into viral particles. When these viral particles are released and infect new cells, the cellular DNA from the prior cell is transferred into the newly infected cell. This process of transduction is, however, not considered of great relevance in the assessment of risks of horizontal gene transfer from GM crops to microbes or humans, as there are only few viruses that can infect both plant and microbial cells, or plants and animal cells. Transfer of recombinant DNA between plant cells is considered inconsequential, in particular as the sequence of the rice and the *Arabidopsis* genomes provides evidence that viral DNA is not commonly transferred to germ line cells that would allow inheritance to subsequent generations.

Most types of cells that can be cultured in laboratories can take up and integrate free DNA by transformation. Transformation can be induced by a cell's manipulation in laboratories. In some microbial species, it is, however, also known to occur spontaneously in nature, given specific environmental conditions. The uptake of DNA from GM crops by microbes, for example, would occur by transformation. This may occur at any stage in the production or consumption of a GM crop: cultivation, transport and storage, processing, consumption, digestion, and subsequent to excretion. Uptake of naked DNA by microbes is most likely to occur where there is a high density of both the DNA and bacteria that are competent to take up this DNA. Highest microbial counts in plant-derived foods are found in food fermentation processes and after food intake during digestion in the large intestine. The mouth micro-flora has also been demonstrated to be amenable to transformation. The availability of plant-derived DNA in most environments is

largely determined by the break down of plant cells and by the stability of the free DNA in the environment. The stability of DNA depends on the environmental determinants, such as acidity, temperature, shearing forces, and the presence of digestive enzymes, light, and oxygen.

Gene transfer by transformation has been studied *in vivo* in the intestinal tract of germ-free animals that were populated with different microbial species. It is expected that gene transfer of recombinant DNA to microbes can occur in the gastro-intestinal (GI) tract, but could, however, to date not be detected; it is therefore likely a very rare event. Gene transfer to a *Pneumococcus* strain was, however, demonstrated in blood sausage (certain *Pneumococcus* species are known to be competent to take up DNA in the presence of serum found in blood).

The transfer of recombinant DNA from a GM crop to a microbe is, however, only consequential if the new the trait is expressed in the microbial cell, and if it is selected for within a population because it confers a competitive advantage.

Gene transfer to human cells

Transfer of recombinant genes to cells of multi-cellular organisms, including humans, may be consequential if the cell's new properties are harmful to the organism, and if the recombinant genes are transferred to germ-line cells so that they are inherited by subsequent generations. Human cells of the gut and the immune system have been shown to take up DNA by endocytosis, a process that involves the folding-in of the cell's outer membrane to form small intra-cellular compartments called endosomes. In cows fed transgenic soybeans, some chloroplast DNA could be detected in blood cells that are part of the immune system. It could, however, not be established whether the DNA had been stably integrated in the genome of these cells (unlikely) or whether they were merely contained in endosomes. In mice fed with large quantities of virus-derived DNA, the DNA could be detected in cells of the intestinal wall, liver, and immune system (spleen cells and leukocytes), as well as in some foetal cells, providing evidence for trans-placental transfer of DNA. The fate of the viral DNA that is single-stranded, circular, and super-coiled, and hence more resistant to digestion, may differ from that of plant-cell contained double-stranded DNA. The experiments are significant as they do provide evidence that DNA that is ingested as part of our diet can be taken up by body cells, that this has occurred throughout evolution, and that this has not been harmful to the development of our (and other) species. Little is known about mechanisms by which germ line cells might be able to take up DNA. This has never been observed to occur in experimental set-ups. The human genome sequence provides evidence that uptake of foreign DNA into the genome of human germ line cells that is subsequently inherited is an extremely rare event that may occur once on the timescale of millennia.

Is gene transfer consequential?

Expression of the newly acquired genes in the recipient cell is a prerequisite for adverse effects of gene transfer. This usually involves transcription of the DNA into messenger RNA (mRNA) that in turn is translated into proteins. Microbial, plant, and animal cells have quite distinct expression systems. Therefore, genes transferred from one cell type to another are often not expressed at all or only at very low levels. Interestingly, at least in currently commercial GM crops, a majority of the recombinant genes, such as the *nptII* gene and most genes conferring herbicide tolerance or insect resistance, are derived from bacteria; transfer of these genes to microbial gene pools hence can be seen to return them to their evolutionary origin. The assessment of risks from horizontal gene transfer hence usually focuses on genes that may confer a selective advantage to microbial cells. These include antibiotic resistance genes, which protect bacteria in environments with the

corresponding antibiotic. If GM crops containing antibiotic resistance genes would contribute to the spread of antibiotic resistance in microbial populations, the potential impact may be the reduction of the clinical value of antibiotics. The risk assessment of antibiotic resistance genes in GM crops is discussed next.

4.2 Risk assessment of antibiotic resistance gene transfer in the human gut

Can transfer of a specific antibiotic resistance gene from a GM crop to micro-organisms increase the spread of resistance in microbial pathogens and thereby compromise the medical and veterinary use of antibiotics (Figure 4-1)? This section discusses the use of antibiotic resistance genes as selectable markers in the transformation of GM crops. The risk assessment and classification of antibiotic resistance genes are into three risk categories. European Community legislation and policies on the use of antibiotic resistance markers are also discussed.

Antibiotic resistance as selectable marker

The use of an antibiotic resistance gene as a selectable marker in cell transformation procedures has been common practice in microbial and in plant genetic research for many years. Selectable marker genes are linked to the trait-conferring gene before transformation to allow identification of the transformed plant cells. The low efficacy of currently routinely used DNA-delivery technologies results in only a very small proportion of targeted plant cells actually integrating the recombinant DNA with the trait-conferring genes stably in the nucleus. Genes that confer resistance to cytotoxic agents, like antibiotics, allow only transformed plant cells to grow on nutrient media that contain the toxic agent; cells that have not integrated the recombinant DNA will usually not grow on these nutrient media. These markers are stably integrated in the nuclear genome of the GM crop; they have no function in the commercial product.

Transfer of antibiotic resistance genes from GM crops to microbes – a rare event

The risk of use of specific antibiotic resistance genes contained in certain GM crops should be judged on a case-by–case basis considering three main factors: the likelihood of transfer of an antibiotic resistance gene from the genome of a transgenic plant to that of a bacterium; the frequency of occurrence of the resistance gene in microbial populations; and the extent of clinical and veterinary use and importance of the relevant antibiotic(s). These factors are considered in turn.

The assessment of food safety of GM crops containing an antibiotic resistance marker has to consider the risk of transformation of microbes in the human gut with this gene. As discussed above, the frequency of transformation of microbes in the human gut is expected to be very low, while the chance of uptake of a particular and functional DNA fragment derived from a plant genome is very small. In fact, it has been estimated to occur once in one hundred million years in the gut flora of an individual; this corresponds to once in ten years for a human population of six billion individuals. Upon digestion of the plant cell matrix, nuclear DNA is released into the gut and can, in theory, be taken up by gut microbes through transformation. All DNA released from plant and other cells in the digestion process competes for uptake, the *nptII* gene conferring resistance to kanamycin is for instance less than 0.000025% of the total maize genome of 2.2 million basepairs.

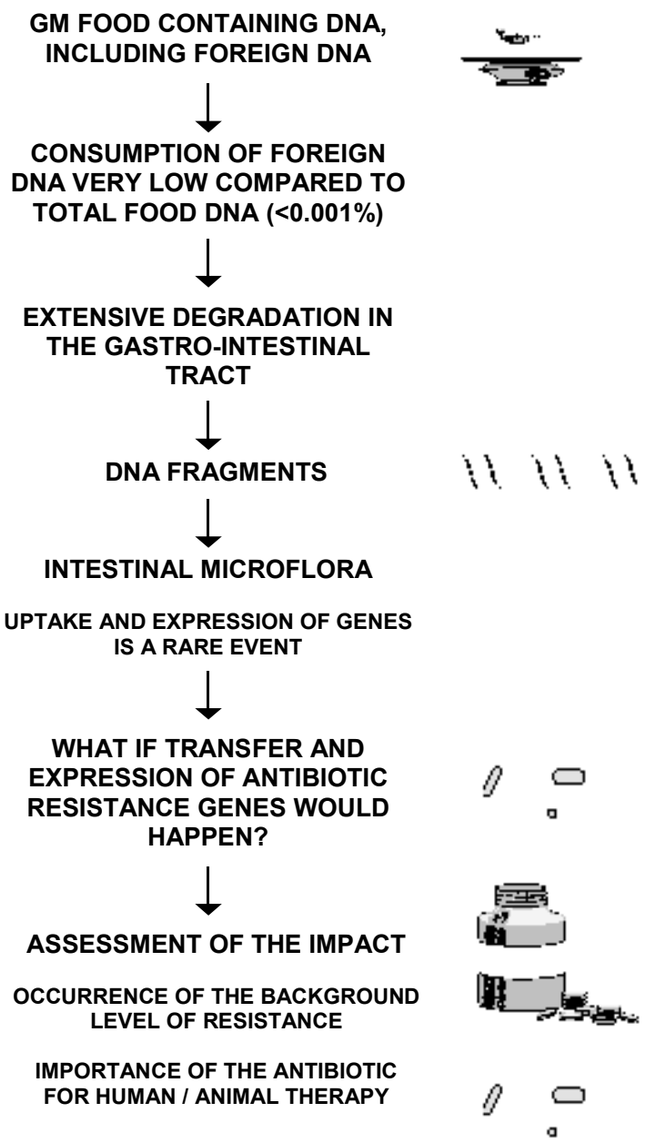

Figure 4-1 Potential gene transfer of antibiotic resistance genes from GM foods to intestinal micro-organisms and its possible impact (designed by G.A. Kleter)

Predicting the rates of uptake of DNA by microbes in the gut is challenging, as the gut micro-flora consists of relative large number of distinct organisms (predominantly less well characterised anaerobes). Little is known about what proportion of these organisms may be amenable to transformation under conditions in the gut. There are therefore considerable uncertainties in the risk assessment. A better understanding of competence of the micro-flora to take up DNA under gut conditions is helpful to reduce uncertainties in the risk assessment; more research in this area is recommended. Experiments in model gut systems have, however, so far failed to provide evidence for the occurrence of transformation of gut micro-flora with recombinant DNA derived from GM crops. Furthermore, some microbes in the human gut contain conjugative plasmids or other easily transferable genetic elements, some of which contain certain antibiotic resistance genes. Genes on such mobile genetic elements are much more likely transferred between gut microbes than taken up by transformation as plant-derived DNA.

Some antibiotic resistance genes are widespread in microbial populations. Diverse species of microbes produce antibiotics to fend off other microbial species that compete for nutrients in the same environment. In what can be compared to an evolutionary arms race, certain species produced antibiotics, while competitors evolved to become resistant. Some of these resistance genes are contained on conjugative plasmids that can rapidly be transferred across microbial populations, in particular in the presence of the antibiotic where resistant bacteria have a competitive advantage. If the recombinant gene used for selection of the GM crop is already widespread in microbial populations in the soil and or human and animal guts, a rare transfer event may not contribute significantly to this spread.

The extent of use of the antibiotic is important in risk assessment for two reasons. First, the type and extent of use determines the selection pressure for the spread of microbial resistance in a clinical or farm environment. The extensive use of antibiotics for medical and veterinary purposes and as growth promoters for farm animals has led to increased spreading of antibiotic resistance genes in the microbial population. Second, the value of the antibiotic is not always proportional to extent of use. Rarely used antibiotics may be saved as last resort on their own or as part of cocktails to combat multi-drug resistant pathogens. If the gene is already widespread in microbial populations in the soil and or human and animal guts, a rare transfer event may not contribute significantly to this spread. If the antibiotic is however very widely used or a tool of last resort, current uncertainties in the risk assessment relating to conditions of competence of a majority of the bacterial species in the gut and elsewhere may not be deemed acceptable. Further research in this area is therefore warranted and the use of such genes should be avoided.

Three risk groups

Antibiotic resistance genes have been assigned to three risk groups based on frequency of occurrence in microbial populations and importance of clinical use of the relevant antibiotics (see also Box 4-1):

- Group I contains antibiotic resistance genes that (a) are already widely distributed among soil and enteric bacteria and (b) confer resistance to antibiotics that have no or only minor therapeutic relevance in human and veterinary medicine. The presence of these antibiotic resistance genes in the genome of transgenic plants is judged insignificant with respect to their occurrence in the environment. The *nptII* gene conferring resistance to neomycin and kanamycin and the *hph* gene conferring resistance to hygromycin are assigned to this group. Genes in this group are considered safe for use as selectable markers.

- Group II contains antibiotic resistance genes that (a) are widely distributed in micro-organisms and (b) confer resistance to antibiotics that are used for therapy in defined areas of human and veterinary medicine. The presence of these antibiotic resistance genes in the genome of GM crops is not likely to contribute significantly to their spread in the environment, but the clinical value of the antibiotic however makes it harder to accept the uncertainties in the risk assessment. This group contains the *bla* gene conferring resistance to ampicillin and structurally closely related drugs, the *aadA* gene that confers resistance to streptomycin and spectinomycin, and the *cat* gene conferring resistance to chloramphenicol. The risk of using such genes use in transformation has to be carefully evaluated on a case-by-case basis.

- Group III contains antibiotic resistance genes that confer resistance to antibiotics relevant for human therapy and therefore should be avoided in the genome of transgenic plants for reasons of a high-standard preventive healthcare. The following antibiotic resistance genes are contained within this group: *nptIII* (conferring resistance to amikacin) and *tetA* (to tetracyclin).

BOX 4-1

ANTIBIOTIC RESISTANCE MARKER GENE CLASSIFICATION

Group I:
- Antibiotic resistance genes widely distributed among soil and enteric bacteria
- Confer resistance to antibiotics of no or only limited therapeutic relevance in human and veterinary medicine: *nptII* and *hph* gene

Group II:
- Antibiotic resistance genes widely distributed among micro-organisms
- Confer resistance to antibiotics which are used for human and veterinary medicine: *bla* and *aadA* gene

Group III:
- Antibiotic resistance genes which confer resistance to antibiotics relevant for human therapy: *nptIII* and *tetA* gene

EU law and policies on the use of antibiotic resistance markers

The recommendation to phase out transgenic crops with selectable markers that confer resistance to clinically used antibiotics has now a legal basis in Directive 2001/18/EC on the deliberate release and placing on the market of GMOs. The concept of "antibiotic resistance markers in GMOs which may have adverse effects on human health or the environment" will be subject of interpretation in the first decisions under the new law, as such markers should be identified and phased out.

4.3 Best practices in the design of GM crops

Several expert committees advising regulatory agencies are discussing recommendations on best practices for the design of GM crops. It is often recommended to minimise introduced recombinant sequences in GM crops. Improvements in methods for crop transformation can simplify the safety assessment of GM crops if they reduce the amount of recombinant DNA and recombinant proteins introduced into the GM crop. DNA transferred to GM crops can be reduced to a minimum by avoiding insertion of multiple copies of recombinant DNA sequences at multiple insertion sites, and by avoiding use of - or removing - selectable marker genes. We build on and refine these recommendations considering the choice of gene delivery technologies, the design of recombinant DNA inserts, and the use of selectable markers in turn.

Gene delivery methods

Improvements in methods for the delivery of recombinant DNA into plant cells are key to minimise recombinant DNA transferred to GM crops. Improvements should aim at ensuring that no DNA sequences other than the trait-conferring gene and the marker gene are transferred and at increasing the proportion of transformed cells that have single simple inserts. Two main delivery systems are used: *Agrobacterium tumefaciens*-mediated plant transformation and particle gun-mediated transformation. In most instances, DNA is integrated into the nuclear genome of the plant cells.

In *Agrobacterium*-mediated transformation, the use of double border vectors, where the "transfer DNA" that is targeted for insertion into the plant genome is flanked by two "border sequences", allows for the transfer of only the necessary genes that are required for expression of the desired trait and for selection of transformed plant cells. The system is imperfect, as the border sequences occasionally are overridden, resulting in the insertion of sequences outside these borders. It is, however, still considered more reliable and more controlled than gene delivery to plant cells using the particle gun. *Agrobacterium*-mediated transformation methods, formerly limited to application in dicotyledon plants, are now also being developed for use on monocotyledon plants (cereals).

Improvements in transformation methodology over the past two decades have resulted in greater control over the DNA sequences that are transferred to the plant genome. Repeated recombinant DNA sequences are undesirable, as molecular characterisation is more difficult, and as repeats may result in recombination and DNA rearrangement at the insert. Gene silencing effects that have been observed where there are several copies of the same recombinant promoter and/or termination signal can introduce uncertainty on stable gene expression. *Agrobacterium*- and particle gun-mediated transformation protocols were developed that, at least in some crop species, yield a higher proportion of GM crops with single simple inserts. However, even with these improvements of plant cell transformation methods over time, the degree of control over the transformation process varies amongst crop species. Physical delivery systems are less controlled than the *Agrobacterium* system in terms of copy number and molecular integrity of inserts. Furthermore, it is important to ensure the purity of the DNA preparation used. Random DNA derived from any bacterium on the workbench may accidentally be co-inserted in transformation vectors or may be shot into the plant genome. It should also be considered that, for instance during bombardment, pieces of plant chloroplast or mitochondrial DNA may be shot into plant nuclear DNA.

Methods have also been developed to deliver DNA into organelles that contain their own DNA, like chloroplasts. One advantage in terms of environmental safety considerations of chloroplast

transformation is that the recombinant DNA will not be present pollen, as these do not contain chloroplasts. Out-crossing can therefore not spread the trait. Another difference is that there are several thousands of chloroplasts in one cell compared to only one nucleus; the copy number of the insert is therefore accordingly increased. Increased copy number on the one hand allows higher levels of gene expression, on the other hand it also maximises the chances of transfer of the recombinant DNA through other mechanisms than pollen flow. Whether this is of consequence in the overall risk assessment of the crop, however, depends on the function of the transferred genes. The increase in copy number has to be weighed against other benefits that can be derived from organelle transformation on a case-by-case basis.

Selectable markers and elimination methods

In spite of foreseeable future improvements in methods for delivery of recombinant DNA, transformation systems for most crops will still depend on the use of selectable markers for some years to come. Several methods are being developed that will allow eliminating selectable markers after the transformation of the GM crop and before the product is marketed. The main advantage of the deployment of such methods is that they allow for reducing the recombinant DNA in the commercially cultivated GM crop to a minimum. Other advantages of marker elimination in product development include the possibility of re-transforming transgenic crops using the same marker gene to insert additional traits. If multiple new traits are combined in one transgenic crop line using conventional breeding methods, the removal of marker genes reduces the risk of gene silencing that can occur due to the presence of several identical gene regulatory or coding sequences and may affect the stability of the expression of the trait. The avoidance of repeated recombinant DNA sequences also reduces the risks of unintended effects from recombination between such sequences. Methods that hold the most potential for allowing routine specific removal of DNA sequences, such as marker genes, from transgenic crops are co-transformation and recombinase-mediated excision.

Homologous recombination relies on the occurrence of base pairing between identical sequences that are in close proximity during the DNA replication process. This can lead to the excision of DNA sequences that are located between the two repeated DNA sequences. At present, recombination frequencies are, however, too low for the routine use of this method in product development to eliminate selectable markers.

Agrobacterium-mediated co-transformation methods rely on creating two separate DNA sequences for insertion on the plant genome, one with the gene of interest, the other one with the selectable marker. The aim is to obtain plant cells in which the gene(s) of interest and the marker gene integrate into non-linked genomic locations. This allows segregation of the gene of interest and the selectable marker by breeding. The method has already been deployed successfully in the development of commercial products. It is, however, much less effective, as in experiments to date, only a maximum of 25% of the transformation events contained single copies of both the marker and the gene of interest in separate genomic locations that are suitable for further development.

Site-specific enzyme-mediated excision systems rely on a recombinase enzyme that specifically cuts DNA at two short DNA recognition sites and then reseals the two DNA strands, leading to removal of all DNA between the sites. Marker genes can be removed from GM crops if they are flanked by the specific recombination target sequences. The likelihood of unintended recombination in transgenic crops developed with this method will need to be assessed.

Expecting all GM crops developed hence forth not to contain markers is, however, premature. Putative novel risks associated with any of these methods have to be weighed against alternatives, such as the use of a selectable marker that remains in the crop. In some cases, an established method may deemed to be preferable to a new method, even if it allows a reduction of the introduced DNA. Many of the methods that promise marker gene elimination still require improved protocols before they can be routine deployed in research and development. Patents restrict access to the sufficiently effective methods, such as co-transformation. The choice of the transformation method should consider the relative safety, cost effectiveness, accessibility, and regulatory and public concerns associated with new methods compared to currently used methods, while research on such methods is encouraged.

Design of inserted DNA

Avoiding the use of DNA fragments that may be transcribed and translated in microbes greatly reduces the possibilities of realising any hazardous effect. Selection of appropriate promoters and transcription termination signal sequences is required in order to avoid recombination events; if the recombinant DNA may be transferred from a GM crop to a virus or a microbe, the presence of multiple virus or microbe-derived DNA sequences on the recombinant DNA may foster genetic recombination between types of DNA that might never have occurred in nature. Gene regulatory elements maximising transcription levels of introduced genes may not always be desirable, as they may also influence expression of genes adjacent to the inserted DNA.

4.4 Conclusions

Gene transfer between organisms is common in nature and has been a driving force in evolution. There is no inherent risk in the transfer of DNA between organisms. The relative risk of gene transfer of recombinant DNA from GM crops to microbes or human cells has to be evaluated with respect to the relative risk of a similar event occurring in nature. The potential impact largely depends on two factors: first, on the function of the transferred DNA in the recipient cell; and second, on whether the recipient cell may have acquired the same gene from a source other than the GM crop.

We deem the risks of gene transfer from GM crops that are currently commercial negligible. Transfer to microbes by transformation is a possibility, but only consequential if a new trait is expressed and confers selective advantage. Uptake of GM crop-derived DNA, including the transgenes by human cells of the gut or the immune system, cannot be ruled out; it is, however, very unlikely that transgenic DNA is stably integrated in somatic cells or taken up in germline cells. Even if it should be taken up, the trait conferred by the gene may not be expressed in human cells.

The risk of use of antibiotic resistance markers for selection of transformed plant cells has to be judged on a case-by –case basis, considering their frequency of occurrence in bacterial populations, the extent of clinical use of the antibiotics to which resistance is conferred, and whether the antibiotic is of importance as a last resort.

We have defined three groups of antibiotic resistance marker genes: those that are suitable for use as selectable markers in crop transformation; those whose use has to be assessed on a case-by-case basis for individual products, and those whose use presents an unacceptable risk or uncertainty to potentially undermine the therapeutic value of specific antibiotics. We recommend that antibiotic resistance markers that have been assigned to Group I, such as the *nptII* gene and

the hygromycin resistance gene, can be used without the risk of compromising human or animal health.

The transformation strategy and the recombinant DNA for insertion into the GM crop should be designed with care. Guidelines on best practice recommend minimising recombinant DNA sequences transferred to GM crops in order to simplify the molecular characterisation and reduce uncertainty on potential genetic rearrangements and unstable gene expression. *Agrobacterium*-mediated gene delivery is considered the most controlled gene delivery system that facilitates obtaining GM crops with single, simple, and hence minimal inserts. The design of recombinant DNA transferred to GM crops should minimise the risks of DNA sequences that might foster independent transfer, expression of the DNA in microbes or viruses, and recombination in bacterial or viral genomes, where possible.

We encourage further research and development on transformation methods and methods for the elimination or replacement of selectable markers, in particular in the public sector. Public access to such methods is important. Improvements in methods for crop transformation aim at simplifying the safety assessment of GM crops by reducing the introduced recombinant DNA in GM crops to a minimum. The risks, costs, and benefits of established and potential new transformation technologies, however, should be systematically compared before novel technologies are officially recommended for broad adoption in product development.

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CHAPTER 5

DETECTION AND TRACEABILITY OF GMOS IN THE FOOD PRODUCTION CHAIN

The EU Regulation (EC) No 178/2002 on General Principles of Food Law (henceforth the General Food Law) establishes general provisions for the labelling of foods and the principle of traceability at all stages of the production and distribution chain in the food and feed sectors. Provisions in the General Food Law are that labelling and product presentation should allow consumers to make informed choices and that consumers shall not be misled. The European Commission's labelling policy provides for consumer information on changes in food due to production processes, even if such changes may not impact human health. The European Commission Directorate General for Health and Consumer Protection (DG SANCO) considers provision of consumer information and choice through labelling and product information important to re-establish consumer trust in the EU food safety system after the BSE and other food safety crises.

In July 2003, the Council of Ministers adopted Regulation (EC) No 1830/2003 concerning the traceability and labelling of GMOs and the traceability of food and feed products produced from GMOs. According to the new regulation, labelling shall be process-based: even highly refined oils that do not contain detectable traces of recombinant DNA or novel protein from GMOs, and hence are not materially distinguishable from other oils not derived from GMOs, will need to be labelled. A system of traceability of GM crops at all stages of the food production and distribution chain is required to facilitate product recall in times of a crisis and the control and verification of labelling claims.

Prerequisites for the implementation of the new labelling and traceability provisions include the establishment of systems for documenting the distribution of individual GM crops in the agro-food chain, as well as analytical methods for verification of this information. Sampling and detection methods for verification of the presence or absence of GM crops require qualitative and quantitative approaches. Qualitative detection methods are required either for high-throughput screening of large quantities of samples or distinguishing approved from non-approved GM crops. A specific area of concern is linking traceability systems across countries and between businesses, as food is exported and imported. The development of such methods requires reference materials and product information for all individually transformed GM crops that are in development or commercialised anywhere in the world. The implementation of labelling and traceability systems hence clearly requires more than just technical tools: international agreements on issues relating to detection and traceability are called for.

In this chapter, we take a critical look at where the scientific community stands in the development of prerequisites to meet the standards for implementation of this regulation. This chapter discusses currently agreed thresholds of levels of GM crops that trigger the labelling requirement, sampling, the development of detection methods, and systems for information management and international agreements required for international traceability systems in turn.

5.1 Thresholds

Quantitative detection of GM crops in food products is necessary to verify compliance with current legal provisions on the 1% threshold of adventitious presence that is deemed acceptable traces of approved materials in foodstuffs that are not labelled. The recently adopted Regulation (EC) No 1830/2003 concerning traceability and labelling of GMOs allows for the presence of up to 0.9% for GM crops that are approved for import into the EU in food and feed items. For GM

material which has benefited from a favourable opinion from the Community Scientific Committee(s) or the Authority, a threshold level of 0.5% will apply for adventitious or technically unavoidable presence. Standardised and validated scientific methods are therefore required for obtaining representative samples and qualitative and quantitative detection methods for GM crops in diverse foodstuffs, including commodities and processed food products.

These very low thresholds present a significant challenge to the implementation of labelling requirements: sampling steps and quantitative detection methods should have high resolution to reduce variance even at such low levels of content.

5.2 Sampling

A very important part of the accuracy of the quantitative detection of GM crops in diverse foods, including commodities and processed foods, depends to a large extent on the sampling protocol. Since in most cases, GMOs are non-uniformly distributed in the bulk food, the variance of GM content across samples will likely represent the major contribution to the overall variance observed in quantitative detection. In general, sampling strategies have to take a wide variety of parameters into account, such as the nature of the sample for analysis, including the proportion of the different GM crops and non-GM crops and their distribution in the bulk. The requested level of reliability of the sampling plan depends on the current regulatory threshold level for the adventitious contamination. Sampling errors are strictly associated with the evaluation of both the risk for the consumer, defined as the acceptance of lots above predetermined limits, and the risk for the producer, defined as the rejection of lots below legal limits. Consequences of error in sampling procedures and hence quantitative assessment of the GM content of foods or commodities can lead to false negatives and false positives: product lots above the GMO-content threshold may enter markets unlabelled, and product lots where adventitious contamination results in a GM content below the specified threshold may need to be labelled. Therefore, the adoption of reliable sampling procedures and the definition of the error related to the sampling procedures are very relevant for all parties involved.

Diverse sampling plans are currently available for bulk products with only some of them specifically designed for GM crops. Recently, the European Network of GMO Laboratories was installed. One of the first tasks of this European Network is to develop definitions of sampling and detection errors and approaches for statistical analysis that can be implemented across the agro-food chain. Given the significant administrative burden from the implementation of current labelling legislation and standards on governments and producers, ENTRANSFOOD recommends to develop diversified sampling plans, more stringent plans for those cases where the safety of the food supply is at stake, and more lenient sampling regimes for other cases.

5.3 GM crop detection and identification methods

The detection of GM crops represents a relatively new area of diagnostics; public and private sector engagement in this area of research has increased rapidly since the implementation of the first labelling requirements based on the detectability of recombinant DNA and proteins from GM crops. Rapid high-throughput methods for DNA and protein methods based on micro-array technologies are being developed. Prerequisites for the development of reliable detection methods are: access to information on all globally marketed GM crops, their inserted recombinant DNA sequence, and reference materials, as well as international co-ordination and inter-institutional ring-testing for the methods' validation.

Methods for the detection of GM crops in commodity shipments or processed foods rely on identifying recombinant DNA or recombinant proteins (see Figure 5-1). Protein-based detection methods have been developed and validated only for very few GM crops. Principally, many methods that focus on fractionation, separation, and profiling of proteins and peptides, such as iso-electric focusing, affinity chromatography, and one- or two-dimensional separation approaches, might be applicable to the characterisation of GM crops when compared to the parental non-transgenic line. Unfortunately, the resolution is often not sufficient or resolved patterns are too complex to clearly distinguish a novel GM crop-derived protein from the protein pattern of its conventional counterpart. Several limitations are envisaged for quantitative determinations of protein-based methods. Since expression levels of introduced traits are tissue-specific and developmentally regulated, protein levels in unknown samples hardly can be compared to those in the reference material used, and an accurate statement is only possible if sample matrices are identical to the reference material or if matched standard materials or standards that have been validated for the matrix are available. It seems therefore likely that the quantitative assessment of the GM crop component will be primarily done by DNA-based, rather than protein-based approaches.

At present, the most commonly used DNA based methods involve amplification of a specific DNA with the PCR technique. Real-time PCR is the most commonly used technology for (subsequent) quantification of the GM crop content. The amount of product synthesised during the PCR is measured in real-time by detection of the fluorescence signal produced as a result of the amplification. Also, new methodologies for DNA-based detection of GM crops have been developed in the past few years, one of the most important developments being the use of the micro-array technology. The main principle of the micro-array technology is miniaturisation. Standard molecular biological or other biochemical methods can be performed on a much larger scale in much smaller volumes. This makes it possible to analyse samples not just for the presence of an individual or a selected group of transgenic or control genetic elements, but to extend the analysis to thousands of probes in a single hybridisation experiment. Increasingly sensitive micro-array systems are being developed in order to allow the detection of few genetic elements within the plant's entire genome. To increase the sensitivity, new array systems are currently in development. An example of a promising new development in this area is the electro-array system where the fluorescently labelled negatively charged DNA fragments are guided to individual spots that are positively charged in order to increase the rate of hybridisation events. Other systems increase the surface where hybridisation may occur considerably by using three-dimensional spot structures, reducing the risk of steric hindrance at the same time, such as gel-based DNA chips, or make use of mass spectrometrical methods based, for example, on the Matrix-Assisted Laser Desorption/Ionization Mass Spectroscopy-Time of Flight principle to analyse large biomolecules like proteins, but also oligonucleotides.

Methods must be validated, quantitative (with little uncertainty or error), and high-throughput, as well as allow for distinction of different transformation events of the same crop with the same trait. Only few of the available detection methods have so far been validated in a collaborative trial according to international harmonised protocols (cf. EC-JRC, 2003). In future, methods will have to be harmonised internationally. Several European and worldwide collaborative trials have been organised and draft European standards by the Centre Européen de Normalisation (CEN) and international standards of the International Standards Organisation (ISO) are already available. The number of GM crops for which methods are available in these draft standards is very limited. In this context, the role of the European Network of GMO Laboratories will be crucial from an EU perspective.

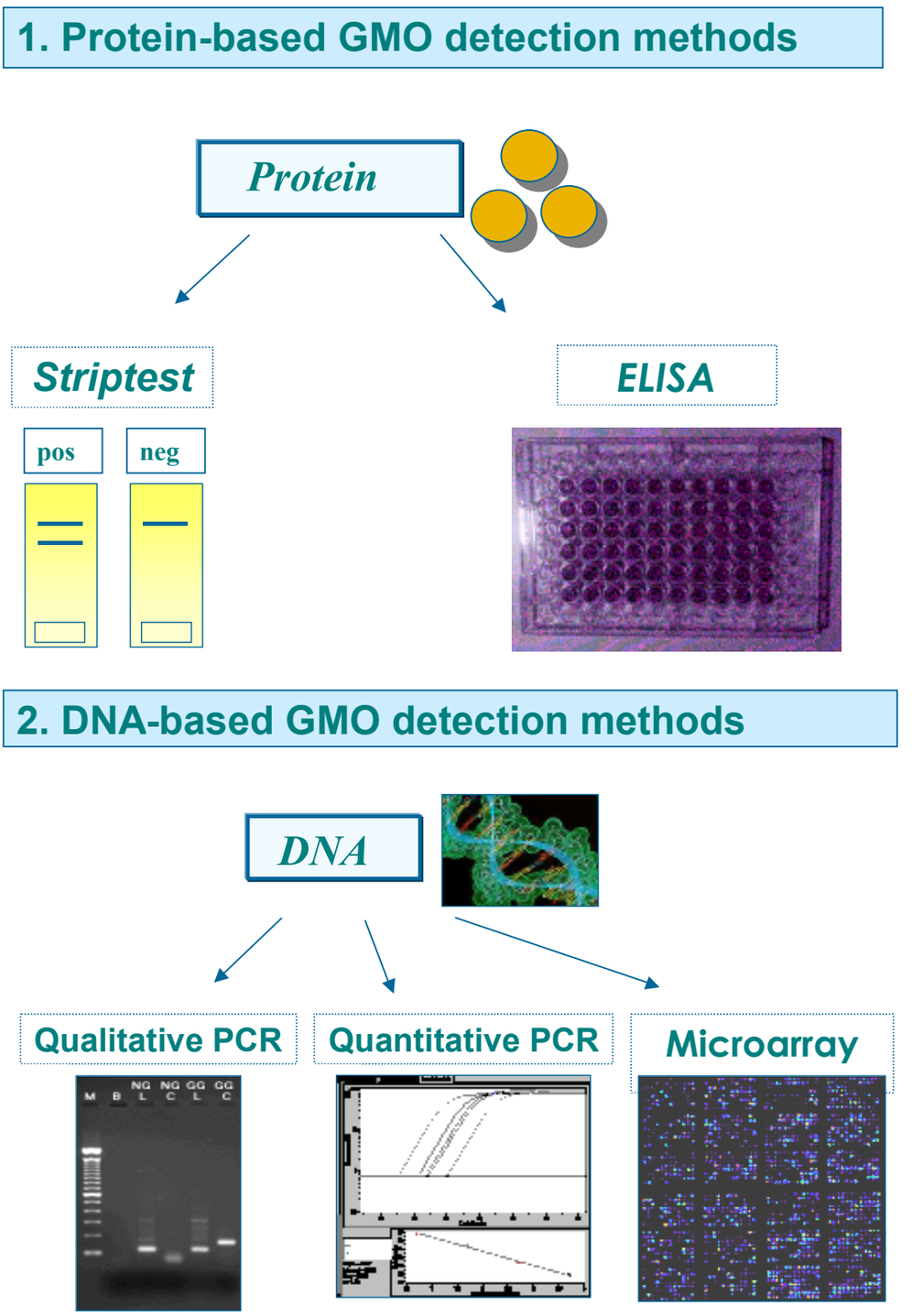


Figure 5-1 GMO detection methods

A key issue in deliberations on international standardisation of methods will be the determination of criteria for method validation, method acceptance, and test reports. The starting point could be a currently constituted working group of Codex Alimentarius Commission (Codex Committee on Methods of Analysis and Sampling) where minimal requirements for the evaluation of data will be agreed. The recently adopted EU legislation on food and feed derived from GMOs addresses the need for reference materials and sequence data: applicants proposing to place a GMO on the market are required to supply reference materials and a detection method to the European Commission. However, the specifications on the types and quantities of materials required, the timeframe in which materials shall be made available, and the type(s) of detection methods requested are still being defined. At present, these issues are being discussed in various fora, including the European Network of GMO Laboratories.

The results of recent research projects and activities in the framework of the European Network of GMO Laboratories have demonstrated that several of the major challenges identified three to four years ago can or have been solved. For instance, detailed characterisation of the transformation event at the sequence level is possible and has been demonstrated for a substantial number of GM crops. However, homo- and hetero-zygous, as well as di-, tri-, and poly-ploid lines will yield divergent quantitative estimates of the GM crop content using any known molecular detection method. It is also very unlikely that the DNA content per weight unit of grains or processed foods produced from different lines is the same, also when they have been subjected to different handling conditions. The analytical steps from sampling to extraction of the sample for analysis (DNA or protein) are believed to contribute most to measurement uncertainty in the final quantitative estimate of the GMO content.

Over one hundred different transformed food crops have been authorised for food and/or feed use globally. And this is only a fraction of GM crops under development and in field trials. As a result of GM crop production and the number of field trials increasing worldwide and seen against the background of the large-scale trade of agricultural goods between countries and continents, a co-mingling with unauthorised GM crops cannot be excluded. Several cases have been reported of GM crops authorised elsewhere, but not in the EU, present in batches grown or marketed within the EU. Authorisation requires risk assessment and approval under EU law. Validated methods to routinely detect or quantify the presence of unauthorised GM crops will not be available in the near future, especially since there is rarely access to sequence information and reference materials. To overcome this problem, deposition of sequence information and reference materials would need to be co-ordinated internationally.

At present, the lack of tools for detection of diverse GM crops released into the environment poses significant challenges for the food industry to comply with EU labelling legislation. Enforcement of the law is equally difficult. Furthermore, EU law requires separate registration of seeds in which two different traits obtained by genetic modification (such as insect resistance and herbicide tolerance) have been combined by breeding. Enforcement of this requirement would also require sampling detection methods that can distinguish between two traits that were stacked into one seed by breeding and two seeds of which each contains one trait. In the analysis of commodity shipments that may contain both types of seeds (single trait and stacked trait seeds), drawing such distinctions is impossible with current detection methods.

Regulatory compliance difficulties exasperate market risks from adoption of agricultural biotechnology, as food products that inadvertently contain unapproved GM crops (that were approved outside of the EU), or that have been inadvertently mislabelled, may need to be withdrawn from the market. Given uncertainties on who is liable and which of these types of

damages are insurable, these laws have direct unfavourable repercussions on the adoption of agro-biotechnology in the agro-food chain.

5.4 Traceability systems

The General Food Law establishes the principle of traceability at all stages of the production and distribution chain in the food and feed sectors. The objective of these general traceability provisions is to facilitate targeted individual withdrawals and/or to provide appropriate information to consumers or control officials. Establishment of a system that allows to trace back to the origin and to understand the distribution of foods requires agreement on at least three of the system's elements: each product must have a unique identifier (a bar code, lot identification number, or container identification marking in case of commodities); guidance must be given on what specific information is recorded; and all points in the production and distribution chain at which this information is recorded must be reliably linked. Audits for verification of the implementation of the system are also required.

Regulation (EC) No 1830/2003concerning traceability and labelling of GMOs contains three main provisions: operators shall have in place a system and procedures to identify to whom and from whom products are made available; operators shall transmit specified information concerning the identity of individual GM crops that a product contains or whether it is produced from GM crops; and operators shall retain specified information for a period of five years and make it available to competent authorities on demand. The regulation does not specify means to transmit and retain this information as the European Commission, in the regulation's introduction on context to the law, holds that existing systems to do so are already in place in many organisations.

The regulation's three objectives, in-line with the General Food Law, are to facilitate withdrawal of products should an unforeseen risk to human health or the environment be established; targeted monitoring of potential effects on human health or the environment, where appropriate; and control and verification of labelling claims. The first objective of allowing effective recall procedures through product tracing can prevent excessive economic losses and/or brand damage. The recent costs exceeding 1 billion US$ incurred through recall of food products that potentially were contaminated with StarLink™ maize and resulting calls for non-transgenic maize supplies demonstrate that the lack of mandatory traceability requirements imposed on all operators in the chain can result in larger than necessary costs associated with loss of trust in the system.

The second objective to facilitate monitoring will only apply to certain future transgenic organisms with attributes that may lead to defined potential health or environmental impacts that can be monitored. One example would be GM crops that are nutritionally enhanced and identity-preserved. Requirements in the regulation do, however, not suffice to allow for post-market monitoring for potential health impacts of GM crops. Where this might be deemed necessary, additional measures to follow the distribution and consumption of foods that contain the GM crops would need to be implemented. This will, in some cases, require transmission of specified information for individual GM crops, which is, however, not mandatory under this regulation.

The third objective is to minimise the burden in terms of testing and sampling of operators. The provision of paper or electronic information on whether transgenic material is present in a food is one way to minimise current testing practices carried out in the European food industry to comply with current food labelling provisions. Sampling and testing for transgenic organisms is mainly required for analysis whenever reliable documentation is not available from exporting countries and for control and inspection purposes. The regulation foresees that the Commission will

develop guidance on sampling and analysis in order to minimise legal uncertainty and alleviate the burden in terms of testing and sampling of operators. Since the first generation of GM crops arrived on the market, the request for non GM crop-derived products has increased in several parts of the world, such as in Europe and Japan, and traceability of GM crops is at this moment mainly related to informing the consumer of the absence of GM crops from specific conventional or organic products.

We recommend considering whether additional testing for verification of claims on GM crop content should not only be carried out at critical control points in the food supply chain. In some cases, paper-based or electronic traceability systems may be adequate (Figure 5-2).

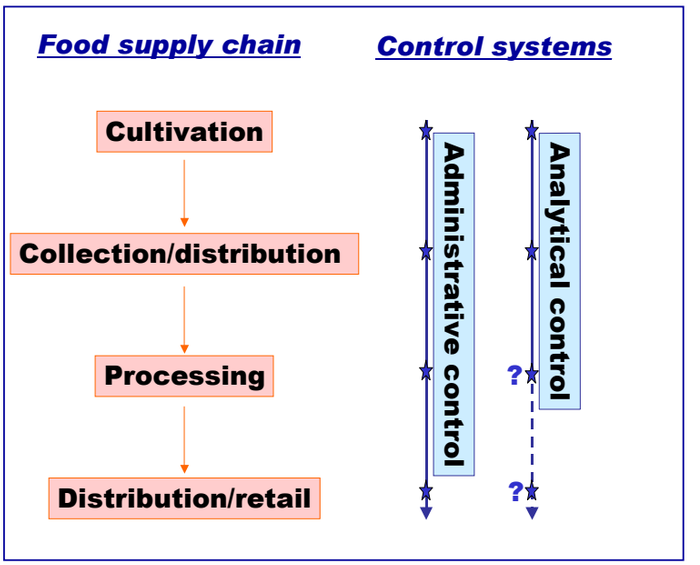
In order to develop an international system of traceability across food production, processing and distribution that takes account of imports and exports of commodities, and processed food products, international co-ordination and agreement on these issues is required. Therefore, the adoption of the proposed regulation on labelling and traceability will have considerable impact on the food and feed chains both at European and at international level. The Codex Alimentarius Commission has therefore recently taken the leadership in the negotiations on definition of standards and guidelines on international systems of product tracing. It is evident that the more marked the differences in regulation of GM crops worldwide in terms of provisions for labelling and number of authorised events will be, the more the impact on the trade of food and feed products will be; this will in turn affect implications and costs of traceability at country level. Diverse labelling provisions and standards across jurisdictions enhance the risk of non-compliance with laws, and the situation of who is accountable for and liable for the resulting damages and financial losses is far from resolved. Diverse regulatory approaches reduce the predictability of such risks and contribute to making insurers apprehensive of insuring such risks.

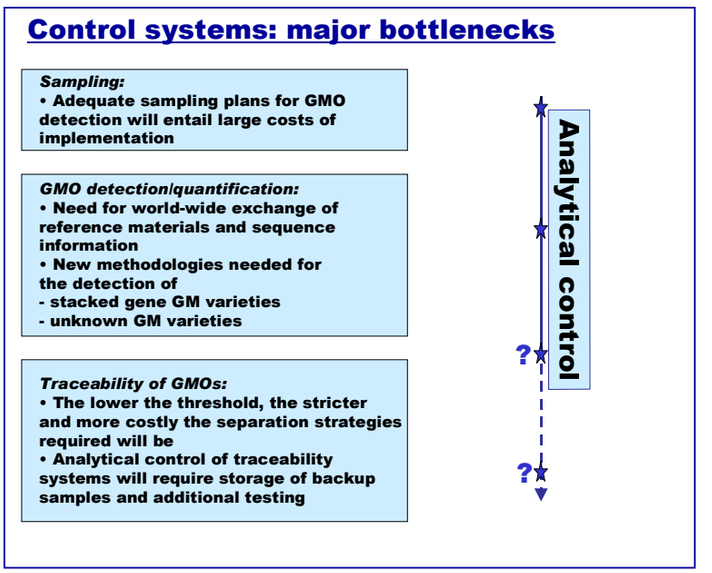
5.5 Conclusions

European labelling and traceability legislation has been established in part as a response to consumer concerns and undermined confidence in the food safety system.

Although some progress has been made to develop detection methods and institutions supervising method development and testing, there are significant technical and political questions that must be addressed before current regulatory requirements can be implemented. Crucial for the development of any GM crop detection method is the availability of DNA sequences specific to the genetic modification, as well as the relevant reference materials. This information is needed for both EU-approved and non-approved GM crops, requiring global exchange of information of individual transformation events on the market and in development. The establishment last year of the European Network of GMO Laboratories is an important achievement towards this goal, but much work remains to be done in a global perspective.

Appropriate sampling schemes for bulk loads where GMO-derived materials may be mixed in a very heterogeneous way will require the analysis of large numbers of samples per load. Reliability and costs of the two steps of the sampling and testing are crucial factors for the traceability of GMOs in this respect. Future improvements in detection methods and sampling plans to optimise traceability systems can be established once agreement is reached at the international level in relation to the availability of information and reference materials of GMO events on the world market and in development.





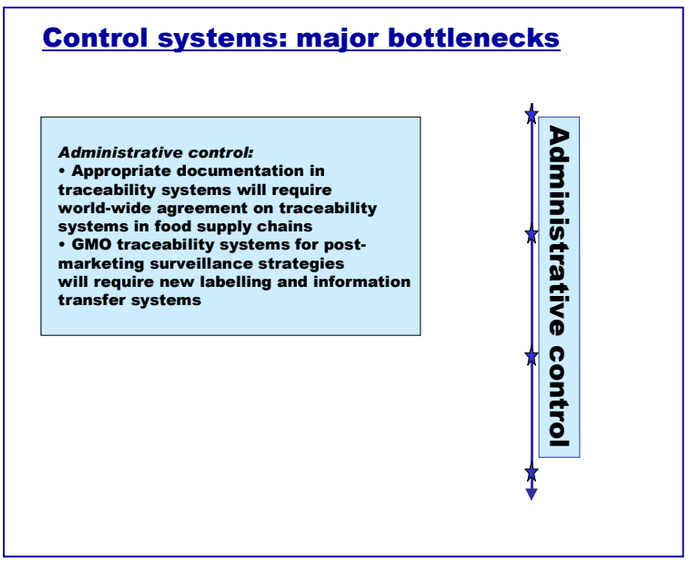


Figure 5-2 Major bottlenecks in traceability systems

Given the burden on administrations and producers, ENTRANSFOOD recommends developing diversified sampling plans, more stringent plans for those cases where the safety of the food supply is at stake, and more lenient sampling regimes for other cases.

Appropriate traceability and segregation systems may reduce the necessity for stringent sampling schemes. The possibility to detect deviations from the documentation in traceability systems may, besides the administrative burden of the documentation itself, require additional measures such as storage of backup samples and additional testing. An appropriate traceability strategy for all GMOs in the food supply chain for the purpose of post-marketing surveillance will require new labelling and information transfer systems. It will be necessary to not only have the information on the label that GMO-derived materials have been used for the production of the food entity, but also which GM events composed the individual ingredients and to what extent.

We recommend considering whether additional testing for verification of claims on GM crop content should not only be carried out at critical control points in the food supply chain. In some cases, paper-based or electronic traceability systems may be adequate.

At present, the lack of tools for detection of diverse GM crops like, for instance, crops with stacked genes poses significant challenges for the food industry to comply with EU labelling legislation.

Traceability represents a valuable tool to face problems related to the introduction of GMOs in food and feed chain and to gain the confidence of the consumer toward this novel food. It is clear, however, that the implementation of any suitable system implies a substantial increase in overall cost of food production that will have to be absorbed by both producers and consumers. This holds true unless a traceability system also helps to save on current costs, such as their use as a tool for more effective inventory- and supply chain management.

The challenge to regulators will be to apply these efforts in the different GMO-related areas, to bundle the expertise, to expand information exchange systems outside the EU, and to come to optimal regulations and standards to suit all stakeholders in order to combine the guarantee of a safe food supply with the consumers' right to informative labelling of food products in the marketplace.

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CHAPTER 6

SOCIETAL ASPECTS OF FOODS DERIVED FROM GM CROPS

6.1 GM foods and the public: What has happened in the past

The first major controversy associated with gene technology in Europe took place in the late 1980s. At this time, there were no products actually being sold to consumers. In 1996, controversy over gene technology was triggered by the arrival of GM soybeans at European harbours. The soybeans, coming from soy plants genetically modified to resist the herbicide *Round-Up*, represented the first large scale marketing of GM foods in Europe. Other applications of biotechnology soon followed: events, such as the attempted commercialisation of GM maize and other commodities, focused public attention on transgenic crops, as did other biotechnology applications, such as animal and human cloning.

The result of these events has been to focus public attention on the emerging biosciences more generally, and on GM foods in particular. The public debate has resulted in the formation of non-governmental organisations (NGOs) with explicit interest in debating the issues associated with the emerging biosciences. There are also increased demands for public participation in the debate about regulation and science, and the rise of the "consumer citizen" who expresses acceptance or rejection of GM products through purchase decisions. Understanding societal responses to GM foods is important if the public and science are not going to disagree about how to proceed with the introduction and commercialisation of new technologies in the future.

6.2 Public acceptance or rejection of GM foods

Public attitudes and risk perceptions

In a democratic society where choice exists, people will not consume foods that they associate with some negative attribute. Various factors may contribute to consumer concerns, for example harm to the environment as a result of agricultural practices, or perceptions that there is uncertainty associated with unintended human or animal health effects. Ethical concerns about production processes are also important. Some technologies may also be described as "transformative", as they have potential consequences for the way in which society is organised. An example might be human genetics, where some people believe that people with certain genes may become unemployable. For this reason, considerable effort has been directed towards understanding people's attitudes towards the applications of biotechnology, such as GM foods.

6.3 Risk perception and behaviour

People's responses to different risks are *socially constructed.* In other words, people's *perceptions* about a hazard influence people's responses to it. Research by Paul Slovic in the United States and elsewhere has consistently demonstrated that the technical risk estimates traditionally provided by experts do not influence people's behaviours and responses in the same way as their risk perceptions. For example, a risk that people perceive to be involuntary in terms of personal exposure is more threatening than one that that is perceived to be voluntary, even if the probability of harm is the same, or possibly even less. People also tend to regard naturally occurring risks are less threatening than hazards that are technological in origin, and they fear potentially catastrophic hazards more than those that affect a similar number of individuals, but at different times. Other concerns are very specific to particular hazard domains, such as food

safety. Public perceptions of risk have often been dismissed on the basis of "irrationality", and have tended to be excluded from policy processes by risk assessors and managers.

Many different methodologies have been adopted to understand what consumers think about GM foods. These have included opinion polls that aim to gauge the absolute level of favour or disfavour in the general public across different cultural and demographic groups (for example, the Eurobarometer surveys, published by the European Commission, see 6.5), to qualitative investigations involving in-depth interviews with consumers. One factor that has emerged as being of great importance in understanding public acceptance of GM foods has been that of trust, whether in regulatory institutions and the motives of scientists, or in information about the risks and benefits of particular technological applications of science and technology. The issue of trust appears to have wider application to the acceptability of risk management activities, as well as social policy more generally. Trust will be considered in the context of the emerging biosciences applied to food production in the next section.

Trust in risk regulation and risk management

There is much debate about the need to increase public confidence in regulation and risk management, particularly in the context of established and emerging technologies. Early efforts at science communication have demonstrated that simply conveying factual information about the risks and benefits of genetic modification (or indeed other technologies and technological practices) is unlikely to result in consumer acceptance of the products of these technologies. More recent attempts to include public perspectives in a direct way within risk policy frameworks begin to assume equality from the public and scientific experts in terms of inputs into deliberations on risk management. Approaches to improving public confidence in risk management processes have included reassessing the way in which risks are managed by regulatory institutions and bodies. Such attempts may focus on increased transparency in regulatory practice (increased tendencies to hold meetings of scientific advisory committee meetings in public, for example) and increased public participation and consultation in regulatory decision-making. The extent to which increased public participation has an effect on public trust in scientific processes and regulatory processes is, however, largely unevaluated.

6.4 Trust in institutions and information sources

Understanding the importance of role of trust has resulted in substantial effort made by some governments, regulatory bodies, and other key stakeholders to direct resource to increasing transparency in decision-making processes. One effect of this has been to open weaknesses in technical practices to public scrutiny (for example, in the case of the different uncertainties associated with risk assessment), increasing the need to develop more effective approaches to risk communication. At another level, there are increased demands to change institutions, to facilitate more socially inclusive decision-making processes *per se*. At both levels, the issue of increasing and maintaining public trust and confidence in institutional, scientific, and industrial practices is highly relevant to the future commercialisation of GM foods, as well as other technological innovations.

6.5 The Eurobarometer results

Five Eurobarometers have been conducted in the last decade to gauge the overall level of consumer attitudes towards biotechnology in the European Union (Eurobarometers 35.1 in 1991, 39.1 in 1993, 46.1 in 1996, and 52.1 in 1999, Eurobarometer 55.2 in March 2002, - Europeans, science and technology). The survey Eurobarometer 52.1 (European Commission, 2000) asked

approximately 16,000 European citizens to indicate their attitudes towards GM foods (Figure 6-1). Participants indicated how useful, risky, or morally acceptable different applications were, and whether their development should be encouraged. Consumer attitudes tend to be more positive about the "first generation" of GM foods, and more negative about the "second generation" of products. When compared to the results of the previous Eurobarometer survey (46.1 in 1996), consumers' attitudes to both generations of GM foods have become more negative in terms of usefulness, moral acceptability, and whether the technologies should be encouraged, but have remained constant regarding risk perception.

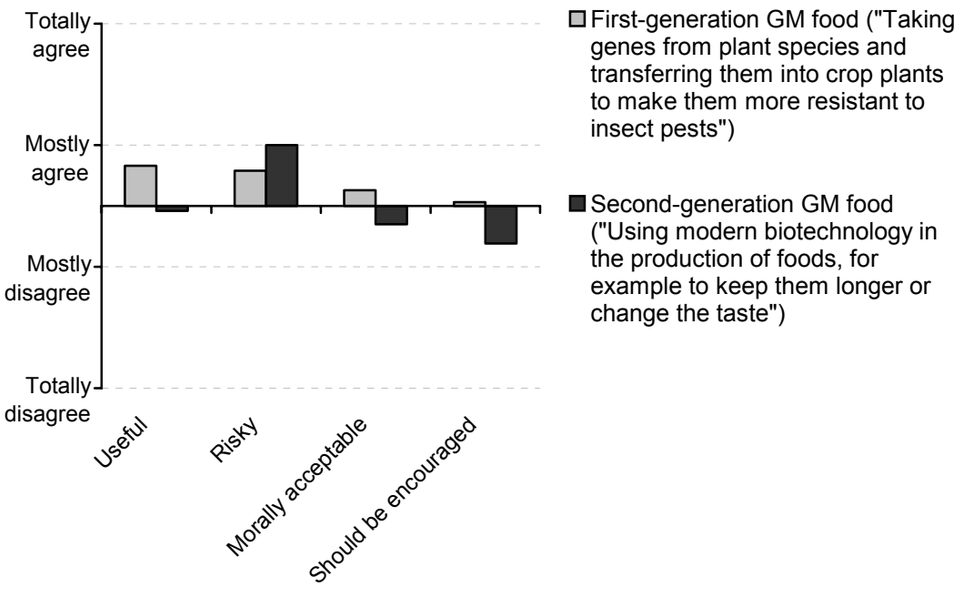

Figure 6-1 Attitudes of Europeans to GM foods (data source: Eurobarometer 52.1).

Consumer concerns

Interviewing and focus groups have been used to understand consumer concerns. Concerns identified in these studies ranged from more or less concrete unintended effects, such as out-crossing and development of super-weeds, to worries prompted by concerns about unintended effects on human health and the environment, and the potential irreversibility of any negative impact. Subsequent qualitative and quantitative research has elucidated the details of moral concerns related to GM foods, 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.

The range of concerns voiced by consumers is remarkably constant over fields of applications. Some of these concerns are intrinsic to the technology itself. Others appear, in part, to be expressions of worries about risks. Perceived unnaturalness is one such example. On the one hand, it appears as an expression of a fundamental concern over human interference with "the Order of Nature". On the other hand, unnaturalness can also be an expression of concern about

risk - people defending this view believe that there are inherent safety mechanisms present in nature and natural processes. Another finding of recent research is that, although the public is concerned with the outcomes of technical risk assessments, they are also concerned about the uncertainty related to these outcomes. People may suspect that risk assessments are based on an insufficient level of scientific knowledge.

Public acceptance of particular applications of genetically modified foods

The supposition that risk perceptions may be offset by perceptions of benefit has led many stakeholders to assume that if a particularly desirable benefit can be developed in the context of GM foods then public acceptance will result. Problematically, how the public defines risk and benefit, and how the experts define the same issues, may be very different. Furthermore, the public is not homogenous with respect to their opinions and attitudes. Differences in perceptions of risk and benefit associated with various hazards exist between different countries and cultures, between different individuals within countries and within different individuals at different times and within different contexts. The food industry will need to predict what kind of GM products will be acceptable as well as beneficial to consumers.

Public demand for information about genetically modified foods

A clear message from technical experts regarding risks and associated uncertainties, including the nature and extent of disagreements between different experts, is helpful. In order to communicate this information to the public, decision-makers must themselves be aware of uncertainties associated with risk assessments, and should therefore have an interest in supporting research to further understand and reduce uncertainties, as well as communicate information about this activity to the public. Research has shown that public distrust in regulators, scientists, and industry is increased if people perceive there to be any uncertainty associated with a particular hazard being hidden by these institutions. There is evidence that regulators and industry have, until recently, assumed the converse to be true – that admitting uncertainty will fuel public distrust in their risk analysis activities! This perhaps explains why public distrust in risk assessment and risk management has continued to increase in recent years. In the case of GM foods, it is important to communicate information about potential unintended effects on human and animal health, and the environment, as well as information about what is being done by scientists and regulators to reduce this uncertainty. Examples might include the introduction of novel allergens into food for human consumption, or examples of potential environmental impact (for example, the case of the monarch blue butterfly in the United States).

For many consumers, labelling is a primary source of information about a food product. From this, one might deduct that the large majority of European consumers would prefer to have information about GM foods available, and an overwhelming majority wants to be able to make an informed choice, necessitating the labelling of GM foods. Moreover consumers expect an assessment of known or potential risks and a precautionary management of these risks before a GM food is granted approval for marketing in the European Community.

The issue of traceability of genetically modified foods and ingredients

As part of the ENTRANSFOOD network, an empirical investigation into the needs of consumers regarding the labelling of GM foods and food ingredients was conducted in three European countries, Italy, Norway, and England. Participants did not believe that a labelling strategy for foods containing GM ingredients could be effective unless accurate traceability mechanisms are developed. Otherwise, there would be no way of identifying where such ingredients are actually

located within the food chain. People in all three countries wanted "clearer labelling of genetically modified foods on food packaging". People approved of enforcing ingredient traceability once they were made aware of its potential. The results indicated that public distrust in GM foods is, in part, driven by lack of personal control over exposure to products, together with reduced opportunities for consumer choice over consumption. Inability to trace GM foods from field to table will act to reduce public confidence in food safety. Failure to implement an effective traceability strategy for GM foods and ingredients may have a negative long-term impact on consumer confidence in safety, particularly in the current climate of consumer distrust in food safety, science and risk regulation.

An alternative view is that, as cross-contamination may also occur between GM and non-GM plants, cross-contamination will occur in the fields and so all foods must be labelled as genetically modified – in this case, of course, improved traceability is unlikely to make any difference to consumer confidence and acceptance.

6.6 Public participation as a way forward

It is often argued that decisions over gene technology should be left to the free market, in so much that consumers can choose to buy, or not buy, GM products. This argument, however, ignores some structural barriers that prevent "the market" serving as a conveyer of democratic decisions. These barriers include factors related to gene technology *per se*, as well as assumptions concerning relations between the market and the consumer.

6.7 The public and policy development

Some people have argued that the concern over GM foods reflects the failure of the authorities and industry to take due account of what was driving public concern. Thus the motives of those developing the framework appeared suspect and unconcerned with public and environmental welfare. Failure to take account of societal concerns associated with emerging technologies may jeopardise the legitimacy of the regulatory framework and regulatory agencies with responsibility for developing that framework. Similarly, the public may also cease to trust industry, which may be perceived to be protecting a vested interest in developing self-regulation regarding technology implementation. However, private companies need to commercialise novel products in the context of appropriate regulation, particularly in controversial areas like gene technology - this is a prerequisite for successful business. Public authorities, on the other hand, must be accountable to secure that the regulation at least to some extent reflects the public views - the alternative being a "technocracy" unacceptable in most countries.

In response to the perceived decline in trust, regulatory bodies have increasingly stressed the importance of transparency in decision making processes, as well as developing mechanisms in order to understand the concerns and values of the general public. One outcome has been an increased interest in participatory procedures to involve the public in the decision making process in some way. This development has in turn led to a growing interest in funding social scientific research into understanding public attitudes to technology (as is the case for GM foods).

There are a variety of pragmatic and ethical reasons for policy-making bodies to involve lay people in decision-making on issues in which the public has a stake. Political theorists and ethicists discuss concepts such as democracy, procedural justice, and human rights, in providing the moral basis for involvement; but it is now recognised that making decisions without public support is liable to lead to public dissatisfaction, dispute, and confrontation. Failure to involve the public is widely assumed to have compromised the legitimacy of governance in policy

development. As a response, there has been increased activity relating to public participation in strategy associated with emerging sciences. Consequently, governmental agencies and independent institutions have played a major role in the development of the participatory tools that may facilitate this development. Examples of public engagement exercises in the area of genetic modification of food and agricultural crops include those sponsored by the Danish Board of Technology (http://www.tekno.dk/index.php3?language=uk), the Rathenau Institute in The Netherlands (http://www.rathenau.nl/uk/default.asp), the Agency for Diffusion of Technological Information in France (http://www.adit.fr/adit_edition/index.php?page), and the Institute for Technology Assessment and Systems Analysis in Germany (http://www.itas.fzk.de/). Examples from the UK have included the Biotechnology and Biological Sciences Research Council Consensus on Agricultural Biotechnology (1994), and, at time of writing, the 2003 public consultation and debate on the environmental release of GM crops sponsored by the Agricultural and Environmental Biotechnology Commission.

This process continues to be taken forward. For example, the European Commission has emphasised the need to introduce "new institutional relationships and forms" reinforcing the process of mutual learning between the public and the scientific community. In particular, the "science and society" agenda identifies the need to change institutional terms of reference and procedures, with the development and use of broader, more inclusive public consultation and dialogue on risk issues and more transparent institutional processes. In the case of transgenic technologies, the policy community has recognised that public concerns about emerging bioethical issues, the potential for unintended effects, and the societal consequences of technology applications are liable to affect public reactions to technology implementation. These societal factors need to be taken into account by policy makers if novel bioscience applications are to reach the commercialisation stage.

Despite the implementation of widespread public consultation processes, there has also been criticism relating to whether public participation makes a difference. Questions have been raised regarding whether the consultation methods are appropriate, and whether the outputs of consultations make any difference to policy development. There is therefore a need for explicit assessment of the impact of public consultation on *policy development*. Consulting the public will be viewed negatively by society if the information resulting from these processes is not incorporated into policy in a transparent and measurable way that can be explicitly evaluated against some criteria of successful implementation. If the output of a public consultation cannot be used, the reasons why should be communicated back to participants and the public more generally.

6.8 Conclusions

Much of the controversy associated with the commercialisation of GM foods has been the result of regulatory bodies failing to take account of the actual concerns of the public, which has fuelled public distrust in the motives of regulators, science, and industry. In part, this may be because communications about food safety issues (including genetic modification) that are based on scientific risk assessments do not reassure the public.

In the case of GM foods, communication efforts have focused on adverse health effects whereas public concern has been much broader, focusing on risk (and risk perception), benefit and need. Risk assessment *per se* does not deal with public concerns. If public confidence in science and technology is to be regained, it is important to explicitly incorporate public concerns into the risk analysis process, through developing new and influential methods of stakeholder involvement (including consumers) and consultation. Once public concerns (and the values on which they are

based) are understood, they can be more effectively dealt with in risk assessment and risk management practices.

BOX 6-1

QUESTIONS TO BE ADDRESSED IN FUTURE RESEARCH

How best should information about what risks are assessed within risk analysis be communicated to the public? How can public concerns be incorporated into this process? How can effective and inclusive public participation in risk management and science and technology policy be developed?

What is the best way to involve the public in the debate about genetic modification of foods? How can any impact of public participation in policy processes be measured?

What changes to institutions need to be made in order to accommodate greater public participation? How should they restructure themselves to make it easier for the voice of the public to be heard?

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CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

The much slower adoption of the technology in Europe demonstrates that rigorous safety assessment by scientists and regulators is necessary, but not sufficient, for gaining societal acceptance of agricultural applications of biotechnology. Introducing a new technology in society requires awareness of who the stakeholders are and what repercussions there may be for each. ENTRANSFOOD has brought together representatives from academia, research centres, biotechnology and breeding companies, food industries, food retailers, regulatory agencies, and consumer groups across Europe to address food safety and societal issues of the introduction of foods derived from GM crops.

Five Working Groups under ENTRANSFOOD addressed the following five topics: Working Group 1 on the safety assessment of foods derived from GM crops developed a systematic approach how to tailor the safety assessment of GM crops to the specific characteristics of the modified crop and the introduced trait. Working Group 2 on the identification and assessment of unintended effects from genetic modification provided an overview on profiling methods and approaches that will help our understanding of crop composition, interaction with environment, and natural variation. Working Group 3 on the assessment of risks from gene transfer across species placed horizontal gene transfer from plants to humans into perspective of nature's other gene transfer processes, and provided recommendations on best practices for the design of GM crops, including on the use of antibiotic resistance markers. Working Group 4 took a critical look at regulation of GM crops including detection, labelling and traceability provisions, standards and their implementation, and assessed prerequisites for implementation of current regulatory requirements. Working Group 5 analysed societal aspects of adopting GM crops in the agro-food chain, paying particular attention to the merits and limitations of labelling foods derived from GM crops to foster their societal acceptance. The main conclusions and recommendations from each working group are reiterated in turn. After detailing ENTRANSFOOD's achievements, we conclude with an analysis of the limitations of the project's structure, scope, and outcomes, drawing lessons for future multidisciplinary deliberation platforms.

The safety assessment of foods derived from GM crops

Safety considerations for foods derived from GM crops are fundamentally the same as those for conventional foods. The safety of widely consumed whole plant-derived foods is a fundamental assumption based on a long-term experience and history of safe use, well knowing that such foods may contain anti-nutritional or toxic substances. Uncertainties in this assessment associated with unintended changes in plant genomes through the insertion of recombinant DNA should always be considered in the light that crop genomes are constantly changing through a broad range of natural and man-mediated mechanisms.

ENTRANSFOOD developed a systematic approach how to tailor the safety assessment of GM crops to the specific characteristics of the modified crop and the introduced trait. The cornerstone of the safety assessment of foods derived from GM crops is the concept of substantial equivalence. This concept prescribes the comparison of the GM crop to a suitable comparator with a long history of use that allows the identification of any significant differences that might impact human health. These differences then become the focus of further analytical, toxicological, or nutritional analyses. The safety assessment of foods derived from GM crops is divided into four steps: characterisation of the parent crop; characterisation of the transformation

process; toxicological evaluation of new gene product(s) and allergenicity assessment; and nutritional and toxicological/allergenicity evaluation of the GM crop/derived food.

ENTRANSFOOD recommends the concept of substantial equivalence as the best available approach to safety assessment of GM crops. The concept is widely deployed to guide the safety assessment by scientists in governments and firms alike. Contrary to what the critics say, guidelines to its implementation are becoming evermore standardised and detailed. For example, the Organisation for Economic Cooperation and Development (OECD) is compiling consensus documents for certain crop species that provide the information considered of most relevance for the characterisation of the parent crop. These consensus documents guide the application of the concept of substantial equivalence.

Discrete substances such as recombinant proteins and metabolites that are newly introduced or the levels of which have been enhanced through genetic modification are characterised by describing what is known about their structure and function. Classic toxicological methods were originally developed for the safety assessment of chemicals in foods, such as food additives. These toxicological methods, including toxicity studies in animals, have been adapted for the assessment of recombinant proteins and introduced metabolites.

Repeated dose studies in laboratory animal species are recommended with recombinant proteins or derived substances to identify potential adverse long-term effects (often, but not necessarily, over a period of twenty-eight days), unless there is sufficient information to confirm the lack of toxicity or pharmacological activity of the recombinant proteins and metabolites, or unless there is extensive experience with the compound, for instance due to a history of safe use or prior approval for human food consumption based on state of the art toxicological studies.

The current approach recommended by experts under FAO/WHO and the Codex Alimentarius for the assessment of the potential allergenicity of GM foods involves five steps: the characterisation of the source organism of the novel protein; the analysis of amino acid sequence similarity of the protein and known allergens; study of physico-chemical properties; where the protein is derived from an allergenic source organism or where there are other indications of potential allergenicity, such as structural similarity to known allergens, these tests are complemented with immunological tests and, where deemed appropriate, further investigations. The possibility of changes in the allergenicity of the whole crop should also be considered. Methods for the assessment of the sensitisation potential of proteins need to be improved, such as through the development and validation of animal models, to allow the transfer of proteins that might share certain structural or physico-chemical characteristics with allergens.

The comparison of the GM crop to its counterpart relies on a targeted approach of parameters indicative of the overall plant metabolism and possible changes from genetic modification that might have health implications. This involves the assessment of composition, physiology, morphology, and performance. Such targeted approaches allow identifying unintended changes from the genetic modification. The focus on key nutrients and anti-nutrients in food crops allows for focussing on identification of those changes in food crops that may have implications for human health.

Animal tests with whole foods derived from GM crops are considered to contribute with useful information to the safety assessment. We recommend such tests should only be a requirement in cases where the composition of the GM food crop differs significantly from that of its unmodified counterpart, or if other tests provide any indications of a potential hazard associated with the genetic modification. In these cases, dietary sub-chronic rat studies (usually, these are of 90 days

duration, assessing the classic toxicological endpoints) are recommended to demonstrate the safety of the food. Further standardisation of test protocols for animal feeding trials is recommended, in terms of design of the diet, when, how, and how often the diet is administered.

The described approach to safety assessment is also applicable to new generations of GM food crops with extensive compositional changes. For GM crops that have been modified extensively such that there is no single crop that is a conventional counterpart suitable for comparison, all new substances or existing substances whose levels have been altered should be assessed on a case-by-case basis; safety studies with the whole crop should also be conducted. The safety assessment of GM crops that are intentionally designed to be compositionally different requires increased attention to two issues: the choice of an appropriate comparator and the estimate of the anticipated exposure. One example of a compositionally altered GM crop currently under regulatory review is oilseed rape that contains lauric acid, a fatty acid not normally found at elevated levels in oilseed rape oil. The product was developed as a substitute for tropical oils (for instance, palm oil) in certain food applications. The comparator with safe use in this case was palm oil.

The issue of long-term effects of the consumption of foods derived from GM crops has been addressed by the FAO/WHO Expert Consultation held in 2000, and was endorsed by ENTRANSFOOD. Very little is known about the potential long-term effects of *any* food, and such effects are difficult to assess at the population level, largely due to the complex and changeable diets that prevent attributing specific health effects to individual food components, and to the genetic variability in the human population. If repeated dose studies with new proteins or metabolites or with the whole GM food would yield indications for potential adverse effects, further toxicological and nutritional studies are warranted before market release may be considered.

Post-market monitoring of foods derived from GM crops is not recommended as a routine practice. It is expensive, sequesters scarce resources for studies of health and food, and is unlikely to provide meaningful information. Post-market monitoring might be considered for identity-preserved GM crops with changed nutritional characteristics in order to confirm the association with a specific health effect: a clear test hypothesis in the form of a causal relationship between food intake and health impact must be formulated.

In summary, it can be argued that the current safety assessment approach allows the determination whether foods derived from GM crops are as safe as their conventionally bred counterparts; in some cases, GM crops are even better characterised than other non-regulated plant-derived foods. In conclusion, the current regulatory requirements and testing regimes are much more rigorous for GM crops than for conventionally bred crops.

Working Group 1 Recommendations:

1. Studies of the structure of plant genomes, functions of individual genes, and a plant's responses to its environment at the molecular level will improve our understanding of the characteristics of food crops that pertain to food safety assessment. Such fundamental research on food crops should be encouraged and publicly funded, as public access to genome sequences of food crops is of paramount importance.

2. The availability of sequence information of entire plant genomes allows the development of micro-array systems to assay induced changes in gene expression patterns. This might, in future, facilitate the assessment of potential unintended effects from the genetic modification, by helping to identify potential changes in gene expression in genomic regions that are adjacent to the insertion locus. The interpretation of such data will, however, be challenging, as a greater understanding of gene functions and changes in expression levels is required before the safety implications of any such change in gene expression can be assessed.

3. ENTRANSFOOD considers of particular value the development of an animal model that would permit the identification and characterisation of potential food allergens. Progress in this area will be facilitated by a more thorough appreciation of the factors that confer to proteins the potential to induce allergy and what distinguishes these from non-allergic proteins. Further research on the structure-function relationship of allergens is encouraged.

4. Advances in genomics and developments of toxicological methods will improve our understanding of health impacts of exposure to various substances in the longer term. The development of genomic expression profiling using micro-array systems prompts research into biomarkers that will allow to assay and compare changes in gene expression upon exposure to specific toxicants and nutrients in different test systems: e.g. cultured cell lines, animal models, and, where appropriate, humans.

Assessment of unintended effects

Uncertainties in this assessment associated with unintended changes in plant genomes through the insertion of recombinant DNA should always be considered in the light that crop genomes are constantly changing through a broad range of natural and man-mediated mechanisms. Uncertainty associated with food safety of GM crops is no greater than uncertainty associated with conventionally bred crops. Unintended effects that alter the composition of food crops are as likely to occur through natural recombination, mutagenesis approaches used in plant breeding, and genetic modification. Variety selection and the assessment of physiology, morphology, and performance for both GM crops and conventionally bred counterparts are sound indicators of unintended effects that may potentially impact human health. Given current regulatory requirements specific for GM crops, they are better characterised than the conventional counterparts we eat. The safety assessment relies on a targeted approach of parameters indicative of the overall plant metabolism and possible changes from genetic modification. Such targeted approaches have proven to be effective for identifying unintended changes that might have implications for human health or the environment.

The application of genomic, proteomic, and metabolite profiling techniques will contribute to improving our fundamental understanding of metabolic and compositional variations of crop plants and of potential alterations in gene expression, protein composition, and associated metabolic consequences that may stem from different cultivation conditions, breeding practices,

or genetic modification. However, profiling methods are not suitable yet for use in formalised risk assessments before sufficient data are available to understand natural variation in different gene expression and compositional parameters of crops plants, and before we are able to assess whether any observed significant changes in gene expression or composition may impact human health. These methods may already be of great value during the research and development phase of GMOs in order to spot potential (intended or unintended) alterations in the composition as result of a genetic modification process.

Working Group 2 recommendations:

1. The allocation of public sector research funds to develop our understanding of food crops, including analyses using genomic, proteomic, and metabolomic profiling methods, is considered important. We encourage the development and validation of profiling methods, as these methods will contribute to improving our understanding of the foods we eat and their potential implications for human health. Such methods will also facilitate the development of GM crops with more complex traits such as nutritional enhancement and tolerance to abiotic stresses.

2. ENTRANSFOOD also recommends the establishment and interconnection for simultaneous searching of international databases on natural variation in gene expression, protein, and chemical composition of crops.

Assessment of risks of gene transfer across species

Gene transfer between organisms is common in nature and has been a driving force in evolution. There is no inherent risk in the transfer of DNA between organisms, since DNA is not toxic. The relative risks of gene transfer of recombinant DNA from GM crops to microbes or human cells has to be evaluated with respect to the relative risk of a similar event occurring in nature. The potential impact largely depends on two factors: first, on the function of the transferred DNA in the recipient cell; and second, on whether the recipient cell may have acquired the same gene from a source other than the GM crop.

ENTRANSFOOD deems the risks of gene transfer from GM crops that are currently commercial negligible. Transfer to microbes by transformation is a possibility, but only consequential if a new trait is expressed and confers selective advantage. Uptake of GM crop-derived DNA, including the transgenes by human cells of the gut or the immune system, cannot be ruled out; it is, however, very unlikely that transgenic DNA is stably integrated in somatic cells or taken up in germline cells. Even if it should be taken up, the trait conferred by the gene may not be expressed in human cells.

The risk of use of antibiotic resistance markers for selection of transformed plant cells has to be judged on a case-by-case basis, considering their frequency of occurrence in bacterial populations, the extent of clinical use of the antibiotics to which resistance is conferred, and whether the antibiotic is of import as a last resort.

ENTRANSFOOD has defined three groups of antibiotic resistance marker genes: those that are suitable for use as selectable markers in crop transformation; those whose use has to be assessed on a case-by-case basis for individual products, and those whose use presents an unacceptable risk or uncertainty to potentially undermine the therapeutic value of specific antibiotics. ENTRANSFOOD recommends that antibiotic resistance markers that have been assigned to Group I, such as the *nptII* gene and the hygromycin resistance gene, can be used without the risk

of compromising human or animal health. GM crops developed with the *nptII* gene and other Group I markers should continue to be approved for commercial use in the EU.

The transformation strategy and the recombinant DNA for insertion into the GM crop should be designed with care. Guidelines on best practice recommend minimising recombinant DNA sequences transferred to GM crops in order to simplify the molecular characterisation and reduce uncertainty on potential genetic rearrangements and unstable gene expression. *Agrobacterium*-mediated gene delivery is considered the most controlled gene delivery system that facilitates obtaining GM crops with single, simple, and hence minimal inserts. ENTRANSFOOD also recommends that the design of recombinant DNA transferred to GM crops should be such as to minimise the risks of DNA sequences that might foster independent transfer, expression of the DNA in microbes or viruses, and recombination in bacterial or viral genomes, where possible.

Working Group 3 recommendations:

1. ENTRANSFOOD encourages further research and development on transformation methods and methods for the elimination or replacement of selectable markers, in particular in the public sector. Public access to such methods is important. Improvements in methods for crop transformation aim at simplifying the safety assessment of GM crops by reducing the introduced recombinant DNA in GM crops to a minimum.

2. The risks, costs, and benefits of established and potential new transformation technologies, however, should be systematically compared before novel technologies are officially recommended for broad adoption in product development.

Detection, labelling, traceability of GMOs, and standards for their implementation

Regulation (EC) No 1830/2003 concerning the traceability and labelling of GMOs and the traceability of food and feed products produced from GMOs requires traceability of foods and food ingredients derived from GM crops and process-based labelling: even highly refined oils that do not contain detectable traces of recombinant DNA or novel protein from GMOs, and hence are not materially distinguishable from other oils not derived from GMOs, will need to be labelled.

We consider that current thresholds for labelling requirements for foods containing or consisting of GM crops are very stringent and pose challenges for implementation. Given the significant administrative burden from the implementation of current labelling legislation and standards on governments and producers, ENTRANSFOOD recommends to develop diversified sampling plans, more stringent plans for those cases where the safety of the food supply is at stake, and more lenient sampling regimes for other cases.

Prerequisites for the implementation of the new labelling and traceability provisions include the establishment of systems for documenting the distribution of individual GM crops in the agro-food chain and analytical methods for verification of this information. Sampling and detection methods for verification of presence of absence of GM crops require qualitative and quantitative approaches. Qualitative detection methods are required for high-throughput screening of large quantities of samples or distinguishing approved from non-approved GM crops. Development of such methods requires reference materials and product information for all individually transformed GM crops that are in development or commercialised anywhere in the world. The

European Network of GMO Laboratories was set up for this purpose; a similar system, however, needs to operate globally.

Sampling plans depend on the quality and nature of the detection method that is used and on the threshold set. Appropriate sampling schemes for bulk loads where GMO-derived materials may be mixed in a very heterogeneous way will require the analysis of large numbers of samples per load. Reliability and costs of sampling and testing depend very much on the test material in terms of the non-uniformity of distribution of GMO-derived material in the sample and the "food matrix" from which DNA or the protein have to be extracted for detection.

At present, the lack of tools for detection of diverse GM crops released into the environment poses significant challenges for the food industry to comply with EU labelling legislation. Enforcement of the law is equally difficult. Furthermore, EU law requires separate registration of seeds in which two different traits obtained by genetic modification (such as insect resistance and herbicide tolerance) have been combined by breeding. Enforcement of this requirement would also require detection methods that can distinguish between two traits that were stacked into one seed by breeding and two seeds of which each contains one trait. In the analysis of commodity shipments that may contain both types of seeds (single trait and stacked trait seeds), drawing such distinctions is impossible with current detection methods.

The three specific objectives of legal provisions for traceability are to facilitate withdrawal of products should an unforeseen risk to human health or the environment be established; targeted monitoring of potential effects on human health or the environment, where appropriate; and control and verification of labelling claims. The fundamental objective is to restore consumer trust through providing information and choice. Traceability of foods and food ingredients, including imported foods, also requires, however, the establishment of international systems allowing traceability of traded foods. It is clear that the implementation of any suitable system implies a substantial increase in overall costs of food production that will have to be absorbed by both producers and consumers. This holds true unless a traceability system also helps to save on current costs, such as their use as a tool for more effective inventory- and supply chain management.

The challenge to regulators will be to apply these efforts in the different GMO-related areas, to bundle the expertise, to expand information exchange systems outside the EU, and to come to optimal regulations and standards to suit all stakeholders in order to combine the guarantee of a safe food supply with the consumers' right to informative labelling of food products in the market place.

Working Group 4 recommendations:

1. Guidelines are required outlining detailed standards for the purity and type of reference materials and additional information on the GM crop that needs to be provided.

2. Closer collaboration between scientists and legislators, in particular in standard setting, is key to ensure agreement on standards that can be implemented and enforced.

3. Given the burden on administrations and producers, ENTRANSFOOD recommends developing diversified sampling plans, more stringent plans for those cases where the safety of the food supply is at stake, and more lenient sampling regimes for other cases.

4. ENTRANSFOOD recommends considering whether additional testing for verification of claims on GM crop content should not only be carried out at critical control points in the food supply chain. In some cases, paper-based or electronic traceability systems may be adequate.

Societal aspects of introducing GM crops in the agro-food chain

The public framing of questions on risk often differs from the framing of scientists. For instance, there is a concern on potential long-term effects from adopting GM crops. Whether society 'needs' the technology is also considered important. Risk assessment does not address these issues and does therefore not address public concerns. If public confidence in the technology and its regulation is to be regained, it is important to explicitly incorporate public concerns into the risk analysis process, through developing new and influential methods of stakeholder involvement and consultation (including consumers). Once public concerns and the values on which they are based are understood, they can be more effectively introduced into innovation strategies, risk assessment, and risk management practices.

Surveys conducted in this project supported that consumer may prefer labelling of products on the basis of both process and product characteristics. Research is needed to determine the most effective form for food labels, which take due account of cross-cultural differences in information preferences, where they exist.

Working Group 5 recommendations:

1. Research is needed to determine the most effective form for food labels, which take due account of cross-cultural differences in information preferences, where they exist.

2. Other questions for further research on societal aspects of GM foods include: How can public concerns be incorporated into this process? How can effective and inclusive public participation in risk management and science and technology policy be developed? What is the best way to involve the public in the debate about genetic modification of foods? How might the impact of greater public participation in policy processes be assessed?

Lessons learnt from ENTRANSFOOD

Deliberations on consequences from introducing biotechnology in society, beyond mere scientific concerns, with representatives from groups that hold diverse sets of values, are a prerequisite for technology adoption. ENTRANSFOOD has provided a platform for such deliberations on food safety assessment, regulation, and citizen's concerns on agricultural biotechnology from a range of different perspectives, including public and private sector scientists, regulators, and civil society organisations.

Given the relatively short duration of the project (3 years and extended with 6 months), the ENTRANSFOOD project's scope was to focus research and deliberations on the food safety of - and societal attitudes to - agricultural biotechnology in Europe. This scope precluded more detailed consideration of three broader themes central to deliberations on adoption of technology: the future of farming, food production, and food trade; the process of risk analysis; and diverse approaches to regulation and distributional effects of regulation within and across jurisdictions. All three themes are central to society's deliberations on agricultural biotechnology and how its introduction should be governed in democracies. Considering the global nature of the agro-food chain, deliberations of each of these themes would benefit from comparing situations in diverse

markets and jurisdictions, not just focussing on the EU. The consideration of ethics and attitudes to the technology by groups with diverse sets of values pertaining to nature and technological progress are also fundamental to deliberations of each of these three themes. These aspects, central to a more comprehensive assessment, need to be considered in future more comprehensive deliberations on the future of agro-food production and agricultural biotechnology.

Other lessons can be drawn from the ENTRANSFOOD project's structure and management. More interaction and more systematic approaches could have been stimulated to get to underlying different values shaping attitudes to technology by ENTRANSFOOD participants with diverse backgrounds. Working Group's 1, 2, 3, and 4 were populated mainly by natural scientists, whereas most social scientists and the permanent representative of consumer groups were clustered in Working Group 5. Participants from WG5 could have contributed to the framing of questions and issues considered in other Working Groups. Timely circulation of draft outputs for comments by members of other Working Groups with the objective to identify cross-cutting issues, and to frame such issues from diverse perspectives, is crucial.

Another inherent disadvantage of the ENTRANSFOOD model was the fact that RTD projects were brought under the umbrella of ENTRANSFOOD after selection and approval by the Commission, which has limited the possibilities to integrate from the beginning to a maximum extent the objectives of the research projects into the overall design and objectives of ENTRANSFOOD. The EU 6-th Framework Programme is designed to avoid these complications.

The participation of non-members of ENTRANSFOOD in the Integrated Discussion Platform Meetings was very helpful, especially of the invited experts, many of which emphasised the need to consider the safety assessment of foods derived from GM crops in the context of food safety assessment in general. The importance of aspects of introducing GM crops in the agro-food chain other than food safety was also highlighted.

Notwithstanding the above mentioned limitations of the ENTRANSFOOD model, the bringing together of different stakeholders in food production using modern biotechnology from a wide range of natural and social scientific disciplines has been a unique experience and has resulted in the development of detailed safety assessment approaches and in new ideas concerning more integrated approaches for risk analysis of new food production technologies, including active consumer participation.

The need for an integrated platform for deliberation on food production and biotechnology

The ENTRANSFOOD project has highlighted the need to continue interdisciplinary deliberations on the introduction of new food production technologies and production practices in order to better understand potential impacts from adoption and diverse approaches to regulation. Four types of questions should be considered more systematically, integrating a wide range of different perspectives: what is the objective of a new product or technology for food production, who will benefit from it, who might be at risk (environmentally, health-wise, economically, or culturally), and how can we ensure that we will learn from the experience?

We recommend the establishment of a Permanent Evaluation and Discussion Platform that explores both scientific and societal issues of alternative practices in food production. Research on improvement of institutional learning on how to govern risks from new technologies and food production practices could be part of its remit, too. Regulators, academics, and stakeholders from the private sector and consumer organisations should work together to map areas where there is

agreement, disagreement, and the need for further research. Such platforms could have several functions, such as organising events to frame questions on risk for expert deliberations, guide the assembly of a knowledge base for experts, review draft expert advice, and review proposed draft regulations and standards. Coordinators of such an integrated deliberation platform should also consider on how they should interact with intergovernmental organisations working on guidelines and policy recommendations on agro-food production and biotechnology, such as the Organisation for Economic Coordination and Development (OECD), Organisations of the United Nations (FAO/WHO), and the European Food Safety Authority (EFSA).

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