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REPORT OF THE OECD WORKSHOP ON THE TOXICOLOGICAL AND NUTRITIONAL TESTING OF NOVEL FOODS

Aussois, France
5-8 March 1997

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NOTE BY THE SECRETARIAT

The work of OECD's former Group of National Experts on Safety in Biotechnology (GNE) led to the production of three reports[1-3] that have been influential in the development of national and international strategies for the safety assessment, not only of foods and food ingredients produced using biotechnology, but also of other new foods.

As a follow-up to the work of the GNE, an *ad hoc* Expert Meeting on Safety Assessment of New Foods was held in Paris in December 1995 to which all OECD Member countries were invited to send delegates. The *ad hoc* Expert Meeting identified a number of work needs which included, as a high priority, a Workshop on Methods for the Toxicological and Nutritional Evaluation of New Foods.

A Steering Group on the Safety Assessment of New Foods met in April 1996 to discuss the proposed workshop and accepted a proposal that the workshop be held at Aussois in France in early 1997. During subsequent discussions it was agreed that, in view of the expertise available and the most immediate needs of Member countries, the workshop would focus its attention on new foods obtained from genetically modified plants. Three goals were identified for the workshop:

- To obtain a better understanding of the role of analytical/toxicological studies in assessing possible unexpected or unintended adverse effects;

- To examine possible approaches through research to develop improved methods for safety assessment that may yield better, more efficient, less costly tests or tests that use fewer animals; and

- To increase mutual understanding of safety testing among Member countries.

Although the Workshop focused on new foods from genetically modified plants it was recognized that many of the topics discussed would be relevant also to new foods derived from other genetically modified organisms and to novel foods from non-genetically modified sources. In addition, the Steering Group agreed that a further workshop would be convened at a later date to examine food safety issues arising from the use of genetically modified micro-organisms.

The Steering Group also discussed other work needs identified at the *ad hoc* Expert Meeting including: allergenicity and the role of serum banks; as well as the role of databases / information management in safety assessment. It was agreed that these needs would be addressed in the first instance through a survey of Member countries. The draft report of this survey formed part of the background papers for the Workshop but is being published separately. A full list of the background papers reviewed by the Workshop together with brief abstracts and the contact details for the authors is attached at Appendix 1. A list of Workshop participants is attached as Appendix 2.)

An earlier draft of this Report was circulated to the Workshop participants. As a result, a number of comments were received which have subsequently been incorporated into this text.

FOREWORD: OPENING REMARKS ON

THE BACKGROUND AND OBJECTIVES OF THE WORKSHOP

by

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Introductory remarks:

On behalf of the French Government, I should like to welcome you to Aussois and to this centre, which is part of France's largest research body, the CNRS. This being said, I must say how sorry I am that Dr. Jim Maryanski of the US FDA, who was initially to co-chair this workshop, cannot be here today for family reasons. You are all aware of the important role that Jim has played in the OECD's meetings on the evaluation of the safety of foods produced through biotechnology. I am certain that you will join with me in expressing our sympathy in the message we shall send him.

I now have the pleasure of introducing Dr. Eric Flamm, a colleague of Jim Maryanski at the FDA, who will be replacing him and co-chairing this workshop with me.

Although the workshop is being held in France, it would not have been possible without the co-operation of Canada and the Netherlands, which also deserve our thanks. More than 70 participants are here today from some 19 OECD Member countries.

Background to the workshop:

It is essential to bear in mind that this workshop represents a new stage in the development of a methodology for evaluating the safety of novel foods, a process that was launched in 1990 at the initiative of the OECD's Group of National Experts (GNE) on Safety in Biotechnology. This group's initiative led to the publication of *Safety Evaluation of Foods Derived by Modern Biotechnology - Concepts and Principles*.

During the same year of 1990, a FAO/WHO consultation took place in Geneva on the same subject, and resulted in the publication in 1991 of a report entitled *Strategies for Assessing the Safety of Foods Produced by Biotechnology*.

These two meetings were the starting point of work that led to the definition of the concept of "substantial equivalence". In 1993 they were followed by a workshop organized by WHO in Copenhagen on the theme of health aspects of the use of marker genes in plants and possibilities for their use in identification and control of genetically modified plants. The following year WHO organized a further workshop, again in Copenhagen, on application of the principles of substantial equivalence to the safety evaluation of foods or food components from plants derived by modern biotechnology. The workshop reports were published in 1993 and 1995 respectively.

In 1994, OECD held a further workshop, in Oxford, on food safety evaluation; the report was published last year.

Finally, a consultation on biotechnology and food safety was organized in Rome in late 1996, in which a number of us participated.

Further information on all these meetings is provided in the report that follows this introduction. I have given this background summary in order to stress the fact that our work during these four days must fit into previous work and take into account what has been achieved internationally. General principles have been defined, in particular the concept of substantial equivalence, which it would be entirely inappropriate to call into question. Our task is not to reinvent the wheel, but to build on those areas in which there is an international consensus in order to improve our approach to the safety evaluation of novel foods.

Thus, following the Oxford workshop, at an ad hoc meeting organized by the OECD, the experts recommended continuing work on food safety evaluation and stressed the need to prepare the ground for a workshop having a more applied objective, i.e. a review of methods and strategies that can be used in the toxicological and nutritional evaluation of novel foods. It is in fact an opportune time to undertake a thorough critical scientific review of the experience gained in a number of Member States that have examined dozens of cases involving the marketing of novel foods. As these primarily concerned transgenic plants or food products derived from these plants, this workshop will be devoted virtually entirely to this subject. However, our conclusions will on the whole be applicable to novel foods in general.

What have we learned from these cases? Were the tests carried out satisfactory, and were they necessary? What additional information would have made the evaluation more effective? What are the weak points of current approaches and what improvements can be made? What kinds of research and testing are required to ensure that consumer safety is adequately protected? These are some of the questions that we shall try to answer during our stay in Aussois.

Organisation of the workshop:

These were the questions that the steering group responsible for defining the workshop's programme took as the starting point for organizing our work.

The first part of the workshop, which will follow this introduction, will be devoted to a review of the previous work by OECD and FAO/WHO and to a presentation of information on the results of recent surveys on serum banks for allergenicity testing and the use of databases.

In the second part we shall compare the methods applied to the evaluation of new plant varieties obtained through traditional procedures with those used to assess plants obtained by means of modern biotechnology. The steering group thought it was essential to address this issue, since the principle of substantial equivalence consists of comparing a new food with a traditional food which is used as a reference and is considered to be safe based on long use without signs of toxicity to consumers.

In the third part we shall examine in detail how substantial equivalence can be established concretely, based on specific analytical or other procedures.

Lastly, in the fourth part the methods used to evaluate the toxic risks and the nutritional impact of novel foods will be presented and discussed, both *in vivo* tests and proposals to use new *in vitro* tests.

This being a workshop, adequate time will be set aside for discussion, and I encourage you to participate actively so that our conclusions will be based on the broadest possible debate.

As our objective is to work on concrete examples, it was indispensable that representatives of the companies that have developed the new plants that we will be discussing participate in this workshop. Many of them are with us here today, and I should like to thank them for having come.

Of course, we shall only be able to work effectively in a friendly and relaxed atmosphere. I am confident that those responsible for the practical arrangements, in particular Peter Kearns of OECD and Eric Schoonejans of the French Agriculture Ministry, have prepared a programme of festivities that we shall all enjoy. I wish to thank them for their efforts.

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SECTION ONE: BACKGROUND

The past activities of OECD's Group of National Experts on Safety in Biotechnology (GNE) have been co-ordinated with those of WHO and FAO in the area of biotechnology and food safety which have included a joint consultation[4] and two more specialized meetings[5,6].

1.1 Previous OECD workshops

In 1992, an OECD symposium on Aquatic Biotechnology was held in Bergen, Norway. Since the earlier report of the GNE had only addressed foods of terrestrial origin, the Bergen symposium considered the potential for the application of the principle of substantial equivalence to foods of aquatic origin. It concluded that, with certain caveats, the principles established for the products of terrestrial biotechnology also applied to aquatic biotechnology.

In 1994 an OECD Workshop was held in Oxford to review the knowledge and experience with respect to methods for the safety evaluation of new foods. The Oxford Workshop reviewed a variety of case studies in considering testing strategies and principles for the safety assessment of foods. The report of the Oxford Workshop elaborated principles for the safety assessment of foods according to three categories: those that are substantially equivalent; those which are substantially equivalent except for the inserted trait; and, those which are not substantially equivalent.

1.2 FAO/WHO Consultation (1996)

In preparation for the development, by Codex (the joint FAO/WHO food standards programme), of guidelines for the safety assessment of foods produced using biotechnology, FAO and WHO convened a consultation on Biotechnology and Food Safety in September 1996. This consultation reviewed national and international (including OECD) activities in the area. It concluded[7] that comprehensive and well enforced food regulations are important in protecting consumer health and that all national governments should ensure that such regulations keep pace with developing technology. It also concluded that since globalization interconnects raw material production to processing and consumers in all parts of the world, it is imperative that proper safety assessments be made of food produced by recombinant DNA (r-DNA) technology, world wide.

The FAO/WHO consultation concluded, in line with earlier FAO, WHO and OECD recommendations, that the food safety considerations regarding organisms produced by rDNA technology are basically of the same nature as those that might arise from other ways of altering the genome of an organism, such as conventional breeding. Since techniques of modern biotechnology introduce no unique safety concerns, it follows that existing approaches to the safety assessment of new plant varieties might form the foundation for the approach adopted for the evaluation of foods from genetically modified plants.

The FAO/WHO consultation agreed with the conclusion of other bodies that the comparative approach embodied in the OECD concept of substantial equivalence is a basic tool in the assessment used to establish the safety of foods derived from genetically modified plants. The consultation recognized that, whilst there may be limitations to the application of substantial equivalence, it does provide equal or increased assurance of the safety of foods derived from genetically modified plants compared to food derived from plants derived by conventional methods. The consultation concluded that there was a need for a flexible approach in determining the reference characteristics for determining substantial equivalence. The determination of substantial equivalence entailed consideration of the molecular characterization of the genetically modified plant, its phenotypic characteristics and key nutrients and toxicants for the plant in question. Although in general, a broader analysis was considered unnecessary, it might need to be considered if there is an indication from other traits that there may be unintended effects of the genetic modification.

In those cases where substantial equivalence apart from certain defined differences is established, the FAO/WHO consultation concluded that the safety evaluation should focus on those differences. Where substantial equivalence can not be demonstrated, the FAO/WHO consultation concluded that it does not necessarily mean that the food product is unsafe, nor that all such products will require extensive safety testing. The consultation advised designing a testing programme on a case by case basis taking into account the reference characteristics of the food in question.

1.3 This workshop

This current Workshop builds on the previous OECD workshops and the FAO/WHO consultation with a focus on applying current experience in order to provide practical help and advice to all those concerned with the safety assessment of foods from genetically modified plants in both developed and developing countries.

The first session of the Workshop reviewed the historical background for the nutritional and toxicological evaluation of food plants and the food safety information typically developed during the process of plant breeding, registration or certification. It also reviewed the data requirements that have been developed for new foods at a national and international level.

The Workshop has reviewed a number of case studies and examined whether there were appropriate data available from traditional food products for substantial equivalence comparison or whether new data had to be developed. The identification of key nutrients, anti-nutrients and toxicants is an important aspect of the determination of substantial equivalence and is considered in detail. Attention has been given to the assessment of unintended effects during a genetic modification and the data required to demonstrate the lack of unintended effects.

The Workshop reviewed the methods that have been used for the nutritional and toxicological evaluation of new foods and in particular those used to address protein toxicity. It also considered new methods and alternative approaches that have been developed and the information needs that are appropriately addressed by *in vivo* studies. Although methods to address protein allergenicity were not within the initial scope of the Workshop, they were discussed, but not extensively.

SECTION TWO: TRADITIONAL APPROACHES TO THE SAFETY ASSESSMENT OF NEW PLANTS

The Workshop considered the historical background for the assessment of food plants through a discussion of the testing applied in traditional plant breeding and variety registration/certification procedures in various Member countries. It is recognized that the major food crops have an extended history of safe use and that the introduction of new varieties of existing crop plants has only rarely resulted in adverse effects in humans.

The objectives of plant breeders include increasing yield, improving quality and improving resistance to stress. As such, testing by plant breeders addresses agronomic characteristics, but not safety *per se*. For certain crops, specific components with safety relevance are part of the testing conducted by breeders due to existing concerns regarding a particular component in that crop (e.g., alkaloids in potatoes, cucurbiticin in squash and zucchini). However, our knowledge of anti-nutritional factors in different plant species is limited.

Variety registration of crop plants is practiced in almost all countries and the European Union. The process of variety registration includes requirements for generation of information on new plant varieties. However, the process is intended to protect the farmer from inferior products, and therefore focuses largely on agronomic qualities with few tests related to safety being included (e.g., glucosinolates and erucic acid in rape seed).

In practice, assessments conducted by the plant breeder for their own purposes and for variety registration procedures have been successful historically in providing new plant varieties which have not resulted in adverse effects in consumers. However, these assessments only rarely address issues directly related to the safety of crop plants and cannot therefore be considered sufficient to establish substantial equivalence or to ensure the safety of a new crop variety. However, the agronomic characterization of a new variety in comparison to an appropriate comparator is a useful tool in addressing the potential for any unintended effects which might be manifested in the agronomic phenotype of the plant.

SECTION THREE: SUBSTANTIAL EQUIVALENCE

Demonstration of equivalence of a new food to an existing food product with an established history of safe use provides assurance that the new food is as safe as the established food to which it was compared. The Workshop concurred with the FAO/WHO consultation recommendation that safety assessment based on the concept of substantial equivalence be applied to establishing the safety of foods and food components derived from genetically modified organisms. Substantial equivalence is established by demonstrating that the characteristics assessed for the genetically modified organism, or a specified food product derived therefrom, are equivalent to the same characteristics of the conventional comparator. The substantial equivalence concept is not a safety evaluation *per se* but it provides equal or increased assurance of the safety of foods derived from genetically modified plants as compared to foods derived by conventional methods. If substantial equivalence is established between a new food and the conventional comparator, no further safety consideration is needed. The concept of substantial equivalence also allows predictions to be made regarding the absence of unanticipated effects of the genetic modification.

Phenotypic and compositional comparisons are useful in determining whether substantial equivalence exists between the novel food and its conventional counterpart or whether there is substantial equivalence apart from certain well defined differences. In the latter case the subsequent safety evaluation will focus on the defined differences. If substantial equivalence cannot be demonstrated, a more extensive safety evaluation may be required which takes into account the properties of the food or food component in question. Several structured approaches have been developed to help determine the information necessary to establish the safety of such foods[8-15].

3.1 Choice of comparator for the determination of substantial equivalence

Comparison between a genetically modified organism, or a product therefrom, and its conventional counterpart for the purposes of establishing substantial equivalence helps to determine whether there has been any untoward effect of the genetic modification and the safety of the genetically modified organism or product relative to that of the comparator. The substantial equivalence comparison is most appropriately carried out on the raw unprocessed crop. However, if the food is limited to a derived processed product (e.g. refined oil) the comparison may be carried out on that refined product processed under conditions equivalent to those used commercially. In all cases it is important that only literature information or data from verified methods are used for the comparison.

When the comparison is intended to examine the possibility of unintended effects of a genetic modification, it is most useful to compare the genetically modified organism with its parent grown under condition that are as near identical as possible. If the genetically modified organism and its parent can be shown to be isogenic apart from the new gene(s), statistically significant differences may be indicative of secondary effects. Subsequently, it can be investigated whether the differences are observed under a range of environmental factors (e.g. climate and soil type).

In the case of many commercial food crops, lines isogenic with the parent line may not be available for comparative purposes and a line that is as close as possible to being isogenic will usually be used. This may complicate interpretation of the results of a comparison since observed differences may arise as a result of a secondary effect of the genetic modification or they may be due to natural genetic variations in the line used as a comparator. The use of several non-modified or control lines may help resolve whether the differences arise from secondary effects or natural genetic variations. Knowledge of the extent of genetic differences between the parent line used for genetic modification and the line used as comparator may also be useful in attributing observed differences.

Statistically significant differences between the genetically modified line and the chosen comparator are not necessarily indicative of a safety concern but they may require further investigation. The differences between the genetically modified line and the comparator should be reviewed against the background of the range of values found in other edible varieties of the crop. If the comparison can be made between lines grown under a range of environmental factors this may help clarify whether observed differences are genetic or environmental in origin. In order to investigate whether an observed difference is genetic or environmental in origin it may be sufficient to repeat the experiment under conditions that are as near identical as possible. If a genetic difference is detected it may be interesting to see what the influence of different environmental conditions is on the observed phenotype. This comparison is particularly valuable when applied to currently available commercial varieties of the crop from the same gene pool and grown under comparable conditions. If the observed value of the chosen parameter(s) for the genetically modified line fall(s) within the range found in commercial varieties of the crop there are unlikely to be safety concerns associated with that parameter.

Where there are significant differences between the modified line and the comparator(s) and these fall outside the ranges for commercial varieties of the crop, unless this is the intended effect, there may be a safety concern. In cases where the difference resulted from the intended effect, this difference would be addressed as an integral component of the safety assessment. The unintended difference(s) may arise from insertional mutagenesis during the modification process or as a result of metabolic effects of the new gene product(s). In order to address possible safety concerns, the nature of the difference and the underlying mechanism will need examination. The direct significance of the change needs to be considered from a toxicological/nutritional perspective. Knowledge of the mechanism provides insight into the potential for other secondary effects that may also require investigation.

3.2 Environmental Factors

There may be interactions between environmental factors (e.g. climate and soil type) and the crop genotype that influence the phenotype and composition. Thus, any variations in environmental factors will need to be taken into account in the assessment of substantial equivalence. Data collected from multiple locations, and during difficult seasons, are useful in addressing the variation associated with environmental interactions. It is particularly useful if the data are representative of the range of conditions under which the plant is expected to be grown. Further work is needed to determine appropriate strategies for assessing the impact of environmental factors on the agronomic and analytical characteristics used for determining substantial equivalence.

3.3 Choice of Component for Comparison

As mentioned earlier, establishing substantial equivalence can be used to demonstrate the absence of untoward secondary effects of a genetic modification and the relative safety of a new food compared to its conventional counterpart. If the comparison shows the absence of untoward secondary effects of a genetic modification, this conclusion will be equally valid in all countries and for applications

of the modified crop. However comparisons showing the relative safety of a modified crop, or a product derived therefrom, compared to a conventional counterpart must also take into account the specific nutritional contribution that the food or food product makes to the overall diet for the population in the country under consideration.

The components compared during the substantial equivalence evaluation are selected on a case by case basis and include key nutrients and toxicants. The more key nutrients and toxicants that there are in a crop, the more components will need to be analyzed and assessed. The selection of the components for comparison will need to take into account the structure and function of the inserted gene(s) and the crop species under consideration. The components to be assessed should include any non-nutritional components with a physiological effect as well as any components that might be predicted to be affected by the new gene product(s) based upon the function of the gene product.

In determining key nutrients, and in determining the consequences of any changes in those nutrients, it is important to consider the effects of any processing that the food might undergo before consumption as well as the potential intake of the food in question by the population at large and by sensitive sub groups. It follows that for a food that is likely to traded internationally, the nutrients considered to be key for the purposes of establishing the safety of a new food relative to its traditional counterpart may vary from country to country.

The number of parameters examined should be limited to those necessary to provide adequate reassurance regarding substantial equivalence. For most major food crops there is a considerable body of experience in determining the parameters that may be useful in establishing substantial equivalence. However, there remains some level of inconsistency in the components being assessed by different developers or being required by regulatory agencies. A consistent approach to the establishment of substantial equivalence would be improved through international agreement on the appropriate components on a crop by crop basis.

3.4 Utility of chemical-analytical fingerprinting

Compositional analysis based on single compounds as a screening method for unintended effects of genetic modification, has its limitations with regard to (unknown) anti-nutrients and natural toxins, especially in less well-documented species. Alternative methods, based on chemical-analytical fingerprinting techniques, or on the detection of altered gene expression by amplification of specific subsets of mRNA, are still under development, and promising results have been obtained. These methods are likely to be more informative than conventional analytical methods, but further validation is required. It should be emphasized that alterations in chemical composition or gene expression do not *per se* imply that the product is less safe, but the toxicological significance of such alterations should be further explored.

3.5 Databases

Databases provide a potentially valuable resource to facilitate the determination of substantial equivalence between a new food and a traditional counterpart. They may also be useful in addressing specific safety issues such as allergenicity. Examples of these data bases include many national data bases of nutrients and toxicants in food crops and gene/protein sequence data bases including data bases on known food allergens. The Workshop noted the preliminary results of a survey carried out by OECD in Member Countries of allergenicity and the role of serum banks and the role of data bases / information management in safety assessment[16]. The Workshop, whilst recognizing the value of these resources in determining substantial equivalence emphasized, however, that they should be used with caution and, in particular, that there is a need to ensure that only validated data are used. It is important to note that since establishment of substantial equivalence is a dynamic concept, the incremental building of information regarding modified crops and their traditional comparators is fundamental to the process. The information developed for both modified and traditional crops should be captured in data bases in order to enhance the information available for future comparisons.

SECTION FOUR: METHODS FOR THE SAFETY EVALUATION OF NEW FOODS AND NEW FOOD COMPONENTS

4.1 Allergenicity

Genetic modification allows the transfer of genes from essentially any organism into any food source organism, including genes that encode food allergens. Therefore, assessment of the allergenic potential of the protein(s) encoded by the transferred gene is essential. The assessment of the allergenicity of genetically modified crop plants has been the subject of recent reviews[17-20]. Allergenicity assessment should be focused on the source of the introduced gene and the nature of the expressed protein. Generally genes from plants known to produce major food allergens should be avoided. However, when the introduced gene, because of its especially interesting properties, is obtained from an organism known to be associated with food allergy (e.g. peanuts), the gene must be shown not to encode an allergen. Sera from individuals documented to be sensitive to that specific food source should be used in validated *in vitro* assays to establish that the transferred gene does not encode an allergen. Negative or equivocal results in *in vitro* studies may be confirmed using *in vivo* skin prick tests with sensitive individuals. For the most commonly allergenic foods, approved challenge procedures under medical supervision may also be warranted. Foods that fail to elicit positive results in adequate and well controlled skin prick or challenge tests should generally be treated like any other food in regards to allergenicity.

When a food contains a gene derived from sources with no history of allergencity, there are a number of factors that can serve as indicators of potential allergenicity including amino acid sequence homology to known allergens, stability to processing and sensitivity to digestibility by gastrointestinal proteases[7]. If there is significant homology to known allergens, sera from individuals sensitive to that food should be used to assess the allergenic potential. For all other proteins, the physicochemical properties of the protein should be compared to the properties shared by known allergens, including resistance to simulated gastrointestinal digestion and food processing conditions that may remove the protein from the final food product. The level of the protein in the final food product should also be considered, since allergens are typically abundant proteins in that specific food. Food containing proteins that do not share physicochemical properties of allergens should be introduced as with foods derived from other new plant varieties.

4.2 Toxicity Testing of Whole Foods

The Workshop considered several examples of new methods and strategies for the safety evaluation of complex foods. Toxicological and nutritional studies may be warranted for new foods which are identified not to be substantially equivalent to existing foods as assessed on a case by case basis depending on the nature of the introduced modification and its potential dietary intake. Application of conventional animal studies as performed for safety testing of single chemicals, food additives, pesticides or veterinary drugs, is beset with many difficulties in the case of whole, complex foods. Compositional dietary problems, the presence of confounding factors, insufficient sensitivity of specific toxicological endpoints and inadequate experimental designs have led in the past to inconclusive answers with respect to the safety of complex foods. Nutritional imbalances may have led in certain cases to adverse effects

not related to the specific properties of the whole food. A major limitation of such studies is the difficulty, or sometimes the impossibility, of applying traditionally large uncertainty factors for extrapolation to safety for humans. However, at the present time, *in vivo* studies may be performed on a case by case basis for non-substantially equivalent products to assess for unknown toxicities, due to a lack of appropriate *in vitro* alternatives. It is essential to custom design studies on the basis of already available information on the safety of the food components, and according to the objectives of the investigation.

The use of *in vitro* methods for safety evaluation of compounds, successfully applied in pharmacological and toxicological research, may offer advantages when the safety of whole foods is assessed. In particular, information may be obtained on general cytotoxic effects, site-specific bioconversion of food constituents and on mechanisms underlying induced toxicity.

The development of an *in vitro* gastrointestinal model simulating the physiology of the stomach and the intestine of monogastric animals and man, is promising. This model may be useful to study the digestibility and bioavailabilty of novel food components, while also allowing the study of gene transfer in this system.

The potential use of *in vitro* models has in many cases still to be demonstrated and validated, since these systems have distinct limitations with respect to cellular organization, realistic physiological conditions, metabolic capacity and long term performance.

Certain *in vitro* methods may be applied in the framework of establishing substantial equivalence in addition to analytical methods, in order to screen for unintended effects in new foods as a result of genetic modification or of the application of novel food processing techniques. Results from such experiments may trigger further toxicological investigations.

An approach which may be of value in some instances is the combination of a variety of *in vivo* and *in vitro* techniques, in order to obtain better assurance of the relative sensitivity of humans compared to the test species. Among other aspects, identification of early biomarkers for (chronic) adverse effects, determination of bioavailability, and physiologically-based pharmacokinetic modeling should be further pursued and their utility for safety evaluation of foods remains to be demonstrated.

SECTION FIVE: CONCLUSIONS AND RECOMMENDATIONS

The Workshop affirmed the conclusions and recommendations of previous consultations of OECD and FAO/WHO regarding the utility of substantial equivalence in establishing the safety of foods and food components derived from genetically modified organisms, and noted that the concept had broader application to establishing the safety of novel foods. The following conclusions and recommendations of the Workshop address considerations in the establishment of substantial equivalence and the application of additional testing where substantial equivalence cannot be established.

i. Substantial Equivalence:

The Workshop concluded that the demonstration of substantial equivalence concept provides equal or increased assurance of the safety of foods derived from genetically modified plants as compared to foods derived by conventional methods. While establishment of substantial equivalence is not a safety evaluation *per se*, when substantial equivalence is established between a new food and the conventional comparator, it establishes the safety of the new food relative to an existing food and no further safety consideration is needed. If there is substantial equivalence apart from well defined differences, the subsequent safety evaluation will focus on the defined differences. If substantial equivalence cannot be demonstrated, a more extensive safety evaluation may be required, which takes into account the properties of the food or the food component in question. When substantial equivalence is demonstrated predictions can be made regarding the absence of unanticipated effects of the genetic modification. The Workshop emphasized the potential value of databases in determining substantial equivalence.

ii. Traditional Plant Breeder Assessments

The Workshop noted that assessments which have traditionally been conducted by plant breeders in evaluating new crop varieties only rarely address issues directly related to the safety of crop plants and cannot therefore be considered sufficient to establish substantial equivalence or to ensure the safety of a new crop variety. However, the agronomic characterization of a new variety in comparison to an appropriate comparator is a useful tool in addressing the potential for any unintended effects which might be manifested in the agronomic phenotype of the plant.

iii. Selection of Appropriate Comparators for the Purpose of Establishing Substantial Equivalence:

The Workshop noted that in addressing the potential for unintended effects of the genetic modification, it is most useful to compare the genetically modified organism with a closely related line and ideally with its parent, grown under the same conditions. The lack of isogenic lines for many commercial crops may complicate interpretation of the results of a comparison since observed differences may be due to natural genetic variations between the modified line and the line used as a comparator. The use of several non-modified or control lines may help resolve whether the differences arise from

secondary effects or natural genetic variations. The significance of any differences between the modified line and the comparator should be addressed against the range of values found in other edible varieties of the crop.

iv. Impact of Environmental Factors:

The Workshop recognized that variations in environmental factors in interactions with genotype will result in variations in crop plant phenotype and composition. In an assessment of substantial equivalence, the impact of environmental factors are therefore an appropriate consideration. Data collected from multiple locations and during different seasons, can therefore be useful in addressing variation associated with environmental interactions.

v. Selection of Components to be Compared:

The Workshop noted that the components compared during an evaluation of substantial equivalence should be selected on a case-by-case basis and would include key nutrients and toxicants, including anti-nutritional factors, associated with the crop under consideration. The more key nutrients and toxicants typically associated with a crop, the more components will need to be analyzed and assessed. The selection of the components for comparison will need to take into account the structure and function of the inserted gene(s) and gene products and the crop species under consideration. The components to be assessed should therefore include any components that might be predicted to be affected by the new gene product(s) based upon the function of the gene product(s). The nutrients and toxicants considered to be key for the purposes of establishing the safety of a new food relative to its traditional counterpart depends on the potential intake of the food and therefore may vary from country to country.

vi. Development of Consensus on Appropriate Components for Comparison:

The workshop recognized that a consistent approach to the establishment of substantial equivalence might be improved through consensus on the appropriate components (e.g., critical nutrients, toxicants and anti-nutritional compounds) on a crop-by-crop basis which should be considered in a comparison. It is recognized that the components may differ from crop to crop. The Workshop recommended that consideration be given by OECD to the development of crop specific documents which could detail the relevant components of a particular crop which should be considered in establishing substantial equivalence. Of additional note, such crop-specific documents would also benefit from linkage to data bases incorporating information on the composition of crop plants in terms of furthering the consistency of comparisons for the purpose of establishing substantial equivalence.

vii. Considerations Regarding Differences Identified in Comparisons:

The Workshop recognized that where differences between the modified line and the comparator fall outside the range of values for a component found in that crop, further investigation is required. The focus of further investigation should be the defined differences and should address the nature of the difference and its underlying mechanism. In addition, the significance of the change must also be considered from a toxicological/nutritional perspective.

viii. **In vivo *testing:***

The Workshop noted that where substantial equivalence cannot be demonstrated, classical animal studies may be considered for further assessment of new substances responsible for the identified differences in the food product. These animal studies should only be applied to address the specific questions for which such studies are the most appropriate means of generating the necessary information. The Workshop further noted that there is a need to gather further information regarding the design of effective animal studies for the safety evaluation of whole foods and their validation. Future work is required to address the need for validated testing strategies for whole foods. A variety of strategies for testing whole foods might be appropriate for consideration.

ix. **In vitro *models:***

The Workshop recognized that where substantial equivalence cannot be demonstrated and further investigation of the food or food component is warranted, in vitro models may be useful on a case by case basis for screening of the food or food components for potential adverse effects and for mechanistic studies. However, since these models are not yet validated, their importance should not be over-emphasized and there is a need for prudence in the interpretation of data from such in vitro studies. Models showing potential need to be identified for validation as part of the future work.

x. ***Allergenicity:***

The Workshop noted that existing methods will help to identify the presence of known major allergens and minimize the risk that they will be introduced accidentally into food as a result of genetic modification. While no specific methods can be used for proteins derived from sources with no history of allergy, a combination of genetic and physicochemical comparisons exist which can be used as a screen. The application of such a strategy can provide appropriate assurance that foods derived from genetically modified products can be introduced with confidence comparable to other new plant varieties.

xi. ***Testing Strategy Development:***

The Workshop recognized OECD's strength and previous experience in elaborating guidance on testing. The Workshop therefore recommended that OECD consider the elaboration of practical guidance on the testing of novel foods based upon the growing experience in Member countries. It is hoped that this could address the types of analytical tests which are used; experimental strategies for assessing the impact of environmental factors (including the number of test sites and the need for locating tests in different countries); testing strategies for non-substantially equivalent products, including the use of validated in vitro screening and animal studies as appropriate.

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APPENDIX 1: ABSTRACTS OF PRESENTATIONS GIVEN AT THE OECD WORKSHOP ON THE TOXICOLOGICAL AND NUTRITIONAL TESTING OF NOVEL FOODS

TOXICOLOGICAL ASPECTS CONCERNING FOOD SAFETY ISSUES

Dr. Kageaki AIBARA
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Japan has two guidelines which relate to toxicological aspects. One is "Guidelines for Safety Assessment of Foods and Food Additives Produced by Recombinant DNA Techniques" and the other is "Guidelines for Safety Assessment of Feed Produced by the Recombinant DNA Techniques" . According to those guidelines, key questions for the assessment for protein toxicity are as follows:

1 Whether or not the protein exists in the final food products (exclusion of the protein during the process)

2 Consumption history of the protein

3 Digestibility of the protein

4 Biological function of the protein

5 Substrate specificity of the protein, if the protein is an enzyme

6 Some animal studies may be required only when the introduced protein is found to be to of toxicological concern.

7 Following animal studies may be required only when the introduced protein is found to be of toxicological concern.

Acute toxicity study
Mutagenicity study
Teratogenicity study
Carcinogenicity study
Chronic study

GLUFOSINATE TOLERANT CROPS: TEST METHODS TO EVALUATE POSSIBLE HEALTH HAZARDS.

Dr. Jan N. BREMMER
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Glufosinate tolerant crops contain a transgene encoding the enzyme protein phosphinothricin N-acetyl transferase (PAT), which detoxifies glufosinate ammonium, the active component in the non-selective herbicide Liberty®.

Diets containing up to 5% PAT did not cause adverse effects in a 14-day toxicity study on rats conducted according to the OECD Test Guideline 407. PAT has no structural similarity to known food allergens, is not stable at temperatures above 50°C or outside the pH-range 5.5-10.

PAT is present at minute concentrations of maximally 10^{-3}% of the total protein content in genetically modified crops. It is rapidly inactivated and degraded in gastric fluid. PAT is a very specific enzyme; it neither metabolizes acetylate glutamate, the closest structural analogue to glufosinate, nor any of the other normal amino acids present in the mammalian body.

Consequently, it is deemed very unlikely that PAT will confer any toxic hazards or allergenicity to glufosinate tolerant crops.

For adequate hazard assessment of glufosinate tolerant crops, first the herbicide and its plant metabolites need to be examined carefully as defined by various regulatory directives (91/414 in EU).

For assessment of health hazards, possibly conferred by the gene product (i.e. PAT), a short term toxicity test (OECD 407) on the gene product is generally best suited for detection of any systemic types of toxicity. It allows a wide range of endpoints to be tested, using an amount of test sample that can (just) be accommodated. Furthermore, various biochemical assays on the gene product are recommended as appropriate to predict how the mammalian organism handles the gene product. Depending on the outcome further testing may be indicated.

Conventional subchronic feeding studies with the gene modified crop are deemed insensitive and of low value for hazard assessment. They should not be part of the standard registration requirement.

Development of an adequate test method for food allergenicity should be considered. In the absence of such a test method, allergenicity should be estimated based on results of the above-mentioned biochemical assays.

BIOSAFETY OF THE AMYLOPECTIN POTATO

Peter BRUINENBERG
AVEBE- Department of Biotechnology
The Netherlands

All starches contain 20% of amylose and 80% of amylopectin. For many applications amylose is undesirable and the starch industry has dealt with this problem by chemical derivatization. To reduce the amount of chemicals AVEBE decided to develop an amylose-free starch potato through genetic modification. The amylose-forming gene GBSS(granule-bound-starch-synthase) was silenced by anti-sense technique.

For environmental approval frost-resistance and glyco-alkaloid contents were determined. For food approval chemical analysis of the starch with additional information on the vector and the genetic modification was sufficient for approval according to Dutch Novel Food legislation. For feed approval of the co-products of starch manufacturing also chemical and information on the genetic modification was sufficient to get approval. In addition to this AVEBE decided to perform a 90-day feeding trial with rats (30% of transgenic in the diet). There was no effect on the wholesomeness of the genetically modified potato as compared to the parent cultivar.

AVEBE concludes that:

- the potato has a long history of safe use;
- chemical analysis gives only data on known compounds;
- sequence analysis of the vector system gives the most meaningful data on safety;
- 90-day feeding trial demonstrated that the amylopectin potato is safe, and hence that the amylopectin potato is as safe as amylose-containing potatoes.

FOOD PLANTS: A BACKGROUND TO BREEDING AND EVALUATION

Peter R. DAY
AgBiotech Center, Cook College/Rutgers University, USA

Conventional plant breeding of most food crops in the USA has conformed to the standard "Generally Recognized as Safe" (GRAS). The common food plant species are assumed to be safe to most people. Safety is assumed to be assured during quality testing by breeders and their colleagues. Products with unusual taste, or effects following ingestion or consumption, are normally rejected. Some crops are tested routinely for levels of toxic compounds before they are released. For example the tubers of new potato cultivars are tested for alkaloid content in Europe. Some examples of the failure of breeders' tests will be presented. Most conventional breeders pay little attention to the relatively uncommon food sensitivities and allergenic responses among consumers since they assume that prudent consumers will avoid those species once they have established that they cannot eat them without ill effects. Some methods used by modern plant breeders, such as genetic transformation, greatly increase the gene pool that plant breeders may draw upon. These methods raise the question of whether the simple GRAS regulations are sufficient.

INVESTIGATION OF GLYCOALKALOIDS IN TRANSGENIC AND CONVENTIONALLY BRED POTATOES.

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Technische Universität München
Lehrstuhl für Allgemeine Lebensmitteltechnologie
Germany

The concept of "substantial equivalence" plays a key role in the safety assessment of genetically modified foods. Compositional analysis is an essential part of this comparison of the novel food to its traditional counterpart. The major focus is on key compounds, such as critical nutrients and naturally occurring toxicants. One of the most prominent examples of naturally occurring food toxicants is steroidal alkaloids in potatoes. In the present study, the content of α-chaconine and α-solanine, the major glycoalkaloids, has been investigated in traditionally bred as well as in transgenic potatoes in order to demonstrate potential impacts of the application of recombinant DNA techniques on these critical compounds.

The alkaloid content of potatoes in inversely related to the size of the tubers. Based on the investigation of a broad spectrum of conventionally bred potato varieties, a mathematical correlation between tuber size and alkaloid content has been established. By means of power regression experimentally determined concentrations can be calculated for a normalized weight (e.g., 100 g), thus allowing the assessment of different potato varieties in terms of alkaloid content independent from the tuber size analyzed.

This combined analytical/mathematical approach has been used to assess the alkaloid content of genetically modified potatoes. Inhibition of amylose biosynthesis (anti-GBSS) by anti-sense RNA expression had no effect on the glycoalkaloid content. However, insertion of an invertase gene from *Saccharomyces cerevisiae* caused a reduction of the concentrations of these critical food constituents.

ASSESSING THE SUBSTANTIAL EQUIVALENCE OF MONSANTO'S BIOTECHNOLOGY PRODUCTS

Roy FUCHS
CENEGEN/MONSANTO
United States

Monsanto has obtained appropriate regulatory approvals and commercialized six different genetically modified food crops to date. Detailed safety assessments, including extensive compositional analysis, has been completed for a total of seventeen different genetically modified food crops. Based on these experiences, the following has been concluded: (1) these seventeen crops were shown to be substantially equivalent to their traditional counterpart; (2) pleiotropic (unintended) effects have not been observed; (3) processed fractions from substantially equivalent foods were shown to be substantially equivalent; (4) progeny and varieties derived from substantially equivalent crops were also substantially equivalent; (5) compositional analysis should focus on the key nutrients and anti-nutrients; and (6) published values are important to interpret any statistically significant differences that are observed between the genetically modified product and the parental variety. A number of important lessons have been learned during these evaluations: (1) validated methods are critical; (2) data for critical nutrients and anti-nutrients are typically available for the traditional bred crops but limited data is typically available for the less important food components; (3) experimental design for field tests are critical for interpretation of compositional data; (4) levels of components varied much greater between field sites than between replicated plots within sites; (5) need to gain agreement on appropriate data with regulators early in the process; (6) there is no clear consensus on the list of critical nutrients and anti-nutrients in different countries; (7) often more data is requested for animal feed approval than food approval; (8) as the number of components evaluated increases so do the number of statistically significant differences due to chance alone; (9) published ranges for components are important to interpret the biological relevance of statistically significant differences; (10) compositional data should typically be focused on the raw agricultural commodity; and (11) animal feeding studies with foods has added little value to assess the substantial equivalence of foods derived from genetically modified plants. We recommend that: (1) OECD develop a global consensus on critical nutrients and anti-nutrients on a crop-be-crop basis; (2) that food that is shown to be substantially equivalent in one country be so recognized in other countries, taking into account any differences in use/consumption; (3) consensus be reached on what compositional results would warrant additional evaluation; and (4) that the questions most appropriately addressed by animal feeding and the critical endpoints for such studies be clearly defined. Finally, it is recommended that OECD continue to take the leadership role in addressing the scientific issues surrounding food/feed safety assessment of foods/feeds derived from novel foods.

ASSESSMENT OF THE ALLERGENIC POTENTIAL OF FOODS DERIVED FROM GENETICALLY MODIFIED PLANTS

Dean METCALFE, James ASTWOOD, Rod TOWNSEND, Hugh SAMPSON, Steve TAYLOR, Roy FUCHS
United States

Numerous genetically modified food crops that add value to me grower, the food chain and the consumer are being introduced into the marketplace. These products undergo an extensive safety assessment prior to their introduction. One key component addressed during this safety evaluation is an assessment of the allergenic potential of the resulting food products. An international consensus, science-based, decision-tree approach to assess the allergenic potential of these products was developed by a collaboration between representatives of the Allergy and Immunology Institute of the International Life Sciences Institute and the International Food Biotechnology Council. The detailed assessment was recently published in Critical Reviews in Food Science and Nutrition; Special Supplement, 1996. The recommended approach relies on a multi-faceted evaluation that considers the source from which a new gene was derived, amino acid sequence comparison of the newly expressed protein(s) to those of known allergens, use of IgE-containing sera from allergenic patients (where appropriate) and/or assessment of key physicochemical characteristics that are typically shared by known allergenic proteins. For genes derived from commonly allergenic sources, in vitro results should be confirmed with appropriate in vivo evaluations. Using this decision-tree approach, food derived from these new plant varieties should be introduced into the marketplace with the same confidence that food derived from new plant varieties developed through traditional breeding has been introduced for centuries.

ABSTRACT TITLE: ASSESSMENT OF POTENTIAL PROTEIN TOXICITY

Bruce HAMMOND, PhD., D.A.B.T.
Monsanto Company

This presentation focuses on the safety evaluation of protein expression products of genes introduced into plants by any method. Proteins are macromolecules composed of amino acids combined in a specific sequence and are natural constituents of plant and animal cells. Thousands of different animal and plant proteins are consumed every day in the human diet. Once consumed, proteins are generally degraded by digestive enzymes into small peptides and individual amino acids. Proteins are not structurally related to chemical food additives, contaminants or pesticides. Therefore, proteins such as enzymes have never been shown to be direct acting carcinogens, mutagens or teratogens when fed to animals or man. As a consequence, it is generally not necessary to test proteins for these toxicology endpoints when exposure to the protein is via the diet. The safety evaluation for proteins should focus on the following questions: (1) is there a history of safe consumption of the protein in food (2) is the protein structurally/functionally related to proteins with a history of safe consumption (3) does the biologic function of the protein raise any safety concerns (4) is the protein closely related to known protein allergens, toxins, anti-nutrients based on homology searches comparing amino acid sequences (5) does the protein share the physiochemical properties of protein allergens (6) is the protein capable of being digested by proteases present in the GI tract (in vitro digestibility tested with gastric and intestinal proteases). The sensitivity and specificity of this test has been validated with known protein allergens that are resistant to digestion whereas non allergenic proteins commonly found in plants are readily digested. Using this assessment scheme, the proteins that Monsanto has introduced into food crops do not raise any safety concerns. Acute toxicity tests are relevant to assessing the potential toxicity of proteins that may be structurally/functionally related to bacterial or plant toxins. Protein toxins such generally act through acute mechanisms and manifest their potential toxicity after administration of a single oral dose in animals at doses ranging from nanogram to microgram/kg body weight. None of the proteins introduced by Monsanto into plants were structurally/functionally related to proteins toxic to mammals. However, acute toxicity tests were required for registration of those proteins with pesticidal properties (e.g. *Bacillus thuringiensis* insect control proteins). Insect control proteins and other proteins such as enzymes introduced by Monsanto into plants were administered as single oral doses to mice at dosages 103 to 106 fold higher than potential exposures from consumption of food containing these proteins. No adverse effects were observed in mice administered these proteins. Similar results have been reported by others who have dosed mice with proteins derived from genes cloned into food crops. If the introduced protein is structurally/functionally related to anti-nutrients, then a more comprehensive safety and nutritional assessment may be needed. In summary, the safety evaluation of proteins introduced into food crops should be based on a case-by-case assessment using relevant tests to assess protein safety.

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SAFETY EVALUATION OF FOOD SUBJECTED TO HIGH HYDROSTATIC PRESSURE TREATMENT

Anthony C. HUGGETT
Nestle Research Centre, and M. Marchesini.

The application of high hydrostatic pressure treatment as an alternative to heat treatment is of interest with regards to maintaining quality during shelf-life. However, the safety evaluation of food macrocomponents which are subjected to a novel food process present s a challenge. We have developed a strategy for the assessment of the safety of food treated by high pressure at 400 bar for min at ambient temperature. The evaluation comprised several steps including the consideration of the unique effects on the test food component of this novel process compared to traditional pasteurization process, an evaluation of effects on major nutrients and toxicants, and a subchronic feeding study in experimental animals in order to confirm the equivalent safety of the novel process with the traditional one. In order to overcome the problem of nutrient imbalance whilst ensuring a maximal consumption of test product, the animal diets were composed of a human-type diet around the test material. The results of the evaluation will be presented together with other new approaches that can be employed to enhance the diagnostic potential of safety assessments of food macrocomponents.

IN VITRO ASSAY TO EVALUATE THE QUALITY OF FOOD

Dr. Kenji ISSHIKI
National Food Research Institute, Japan

We have been applied in vitro bioassay to evaluate the quality of food. We tried some kinds of assay procedure. We have developed a chemiluminescent cytotoxicity assay and applied it to various kinds of foods and some toxins. This assay was sensible and rapid, so that it would be useful as a tool provides information of food safety.

DETECTION OF ALTERED GENE EXPRESSION BY MRNA FINGERPRINTING

E.J. KOK, A.M.A. Van HOEF, J. KEIJER and H.A. KUIPER
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In the last few years reports have been published by national and international advisory committees on the subject of food safety strategies for plant products derived from transgenic sources. In many cases the reports advocated that in order to improve food safety strategies for complex plant products, it would be necessary to develop new toxicological concepts based on analytical systems in combination with in-vitro systems. Additional toxicological methods should be applied in those cases where data generated in that way would not supply sufficient information on the safety of the product.

It is generally acknowledged that composition analysis has important limitations. In many plant species the information on their composition is limited, especially with regard to the anti-nutrients and natural toxins. Because of this, composition analysis as a screening method for unintended effects of the genetic modification is often severely hampered. It is therefore necessary to develop alternative analytical methods that are more informative in those cases. In this presentation a method is described that aims to detect altered gene expression by means of mRNA fingerprinting or RT-PCR. The method is based on the amplification of specific subsets of the mRNA population. The amplified fragments are then made visible by means of gel electrophoresis. The method is still under development and requires further validation, but has shown to be reproducible and able to detect important differences in gene expression. Future work will focus on the sensitivity of the method. In those cases where altered gene expression is detected it will be important to establish the toxicological significance of the alteration. Alterations in gene expression do not imply that the product is less safe, but significant alterations may point at undesirable changes in the physiology of the plant as a result of the genetic modification. At a later stage the method may become useful to plant breeders as a part of their breeding and selection scheme, especially to select for changes in qualitative traits.

METHODOLOGY EMPLOYED TO ADDRESS PRODUCT CHARACTERIZATION AND TOXICOLOGY FOR PLANTS EXPRESSING PLANT-PESTICIDAL TRAITS.

John L. KOUGH
U.S. Environmental Protection Agency
Office of Pesticide Programs
United States

The methods used by companies to characterize their newly introduced pesticidal traits are presented. To date all plant-pesticides registered for use are proteins so the methodology utilized to describe these traits depends on classical protein chemistry such as amino acid sequence, molecular weight by gel electrophoresis and immunorecognition. The toxicity of the individual proteins has been assessed by both *in vitro* digestion tests and acute oral toxicity. Acute oral toxicity done at 2-5gm/kg insures results of *in vitro* digestion tests. This method assures that the expression level of the individual proteins should not present a problem for human health. Proteins expressed at low amounts are not *a priori* expected to significantly alter nutritional considerations of the resulting food.

The safety assessment deals only with pesticidal trait. The plant is assumed to be substantially equivalent otherwise. Expression level data is not required except to verify claims of lack of food exposure. Otherwise bioactivity is the measure of expression for the pesticidal trait. Assessment of toxicity of the pesticidal protein is done with a purified or enriched preparation at high doses to eliminate the need to consider safety factors.

A suggestion was made to begin to approach pesticidal proteins on a broader scale like has been done for allergens. Toxins and venoms are well known if not always sequenced. Few of these toxins are active by the oral route. Homology similarity could facilitate the assessment if the appropriate algorithm is used to determine hazard.

THE PROS AND CONS OF CLASSICAL AND ANALYTICAL TOXICITY TESTING OF NOVEL FOODS

H.A. KUIPER

State Institute for Quality Control of Agricultural Products (RIKILT-DLO), The Netherlands

The development of safety testing protocols for man-made chemicals like pesticides, drugs, cosmetics and food additives has made it possible to introduce and regulate these compounds in the environment. These protocols comprise physico-chemical analysis, animal experimentation, in-vitro testing, and occasionally human studies. When data from animal experiments to humans are extrapolated, a number of uncertainties have to be taken into account and therefore relatively large safety or uncertainty factors are normally applied, which provide sufficient margins of safety for the consumer. International organizations like FAO, WHO, IPCS and OECD have contributed significantly to the development, standardization and harmonization of test protocols. Traditional safety testing protocols are far from ideal to test the safety of whole foods or complex food components. Dietary problems, confounding factors, insufficient sensitivity for specific toxicological endpoints, and small safety margins have been recognized as serious disadvantages when animal feeding studies with food products are performed. Alternative approaches should be explored using a combination of specific animal experimentation, in-vitro testing and, if possible, human studies. These studies should be focused on the identification of early biomarkers of adverse effects due to chronic exposure, on the description of the metabolic fate of relevant food components and on the determination of the bioavailability of these components. In particular the use of in-vitro systems derived from animals and humans may contribute to a better and more reliable extrapolation of animal data to humans. Aspects of tolerability, digestibility and bioavailability may be studied in human volunteers. Moreover, application of kinetic modeling based on physiological parameters provides promising tools to evaluate the safety and nutritional status of novel foods.

SAFETY ASSESSMENT OF HIGH OLEIC ACID TRANSGENIC SOYBEANS

Mary E.H. LOCKE
DuPont Agricultural Products
DuPont Experimental Station, United States

DuPont Agricultural Products has developed, and field tested since 1995, a new transgenic soybean line which consistently produces an oil with a relative abundance of oleic acid exceeding 80%, *versus* 24% found in typical conventional soybean oil. This soybean line, called high oleic acid transgenic soybeans, is homozygous for a GmFad2-1 cDNA in the sense orientation under the control of a seed-specific promoter. The inserted GmFad2-1 cDNA is causing a coordinate silencing of itself and the endogenous GmFad2-1 gene. In developing soybeans, the second double bond is added to oleic acid in the δ-12 (n-6) position by a δ-12 desaturase, encoded by the GmFad2-1 gene. A second Fad2 gene (GmFad2-2) is expressed in all tissues of the soybean plant. Suppression of the GmFad2-1 gene in developing soybeans prevents the addition of a second double bond to oleic acid, resulting in greatly increased oleic acid content only in the seed. With the exception of the beneficial changes in the high oleic acid transgenic soybeans, the nutrient content of the new beans is well within the range of commercial soybeans.

INDIVIDUAL NUTRIENTS - WHEN TO CARE ABOUT CHANGES

Heddie MEJBORN
National Food Agency of Denmark
Division of Nutrition

The principles for evaluation of new foods are all derived from the concept of substantial equivalence. Already at this point it is important to decide which nutrients to concentrate on, as a complete analysis (all known macro- and micronutrients) will be both unnecessary and very costly.

The information needed will to a large extend depend on the nature and complexity of the new food and the expected consumption level. Initially, the importance of the food for the average consumers nutritional status must be stated, but also vulnerable subgroups must be considered. This brings us to focus on two points:

1) is the content of one or more individual nutrients in the food high?

and/or

2) is the consumption of the food considerable?

Generally, very few foods contribute substantially to the total intake of any individual nutrient. Exceptions are seen, though. In Denmark for example, potatoes provide an important part of the total vitamin C intake for the average consumer (35 %), not because of a high vitamin C content (20 mg/100 g) but because of a high consumption (140 g/day).

The natural content of nutrients in many foods can vary to a great extent and depends on many factors, as for example for fruits and vegetables: cultivars and growth conditions (climate, fertilizer, water).

What must be discussed then, is what criteria are to be set for acceptance/rejection:

- is it a specified change (for example 20%) in content of individual nutrients no matter what the intake of the food is ? - or -
- is it a specified level of contribution to total daily intake ? - or -
- will just a statistical significant difference in nutrient content between the new food and the traditional food be of importance ?

The presentation will discuss these questions based on case studies.

PRESENTATION OF CASE STUDIES IN FRANCE

Antoine MESSÉAN
CETIOM
France

Since 1986, the French biosafety committee (Commission du Génie Biomoléculaire) gave consent, for more than 400 research applications representing more than 3000 release sites. Furthermore, 13 applications for marketing have been reviewed. From this experience of novel foods mainly based on genetically modified plants, some lessons can be drawn and the lecture focuses on those facts we consider as relevant regarding the evaluation process for safety and nutritional issues.

The general principles applied by the French authorities for evaluating the GMO's can be summarized as follows :

- products derived from modern biotechnology must not only improve productivity and quality but also the security for consumers,
- risk assessment is performed on a case by case basis and addresses various concerns through an analytical approach,
- socio-economical context is an important issue to be taken into account,
- introduction of genetic material should be as limited as possible (restricted when possible to those traits which are desirable). Thus, unexpected effects of genetic transformation should be reduced and one could save some safety studies.

Although we reviewed a large number of applications, only a few number of crops and traits have been considered for marketing release: mainly corn and rapeseed as crops, herbicide resistance and insect tolerance as traits. Many other crops and traits are currently under development and new concerns will certainly be raised in the very near future, e.g., role of feeding experiments for assessing food safety of non transformed products.

With respect to the substantial equivalence assessment, the dossiers submitted for marketing reflect a great heterogeneity when taking into account the variability due to genetic and environmental factors; either global comparison is made with published ranges of variation or a specific comparison between a transgenic line and its isogenic counterpart is provided on a site by site basis. When available, this direct comparison is useful in order to assess expected pleiotropic effects of genetic transformation.

Allergenicity issues are usually addressed through a general comparison of properties of the protein encoded by the transgene with properties of known allergens. Standardized databases and assessment methods are thus required.

In order to assess toxicology, protein expression is generally measured in different plant organs and at different stages. However, data provided appear to be quite heterogeneous. Furthermore, most of

the dossiers contain an acute toxicological study but feeding experiments are not always carried out. Moreover, the protocols used differ slightly between the applications. Those experiments, when available are good indicators of the actual impact of analytical differences or of unexpected effects on food safety.

To sum up, as we have still little experience and rather heterogeneous methods for assessing novel food safety, we claim for more standardized approaches and for sharing data and experience within OECD.

CHEMICAL FINGERPRINTING AND *IN VITRO* TOXICOLOGICAL PROFILING FOR THE SAFETY EVALUATION OF TRANSGENIC FOOD CROPS

H.P.J.M. NOTEBORN
Rikilt-Dlo

The introduction of genetically modified food crops has set forth three central questions with respect to food safety, which are the toxicity and allergenicity of the newly introduced proteins, and the potential of secondary metabolic changes in the crop due to the insertional event that may compromise food safety. Application of the concept of substantial equivalence is a basic tool in the assessment used to establish the safety of novel foodstuffs derived from transgenic crops. It is a dynamic, analytical exercise in the assessment of the transgenic food plant relative to existing comparators. This presentation is focused on method development for the comparative screening of transgenic BT-tomatoes for their chemical and toxicological profile taking into account the reference characteristics of appropriate counterpart(s) and environmental factors. Investigations which should lead to a redesign, refinement or even replacement of (sub)chronic rodent feeding trials. Thereto, a tiered approach is proposed focusing on: (i) analytical fingerprinting analysis in order to identify unintentional and/or undesired changes in the overall chemical composition of the plant product; (ii) *in vitro* toxicological profiling using early biomarkers in order to identify interspecies differences and the toxicological relevance of differences found in their chemical composition, and (iii) if the preceding testing indicate a potential safety concern, rodent studies with human-type diets may be appropriate.

For the purposes of this exploratory study transgenic tomatoes (varieties: San Marzano and Moneymaker) expressing a *Bacillus thuringiensis cry1Ab5* gene were used, and simple homogenization and extraction steps were considered a reasonable starting point. With respect to chemical fingerprint analysis the applicability of Liquid Chromatography (LC) combined with high-resolution one-dimensional proton-Nuclear Magnetic Resonance (NMR) was explored. This approach is not a safety assessment per se but an empirical comparative analysis of differences in the overall composition of constituents with MW < 10 kDa relative to those of existing components in that crop. Three different comparisons of populations will be discussed, namely, isogenic lines grown under identical environmental conditions, isogenic lines grown at multiple locations, and the transformant and the range of that crop. Reproducibility was under control and the spread due to sample preparation, fractionation, software processing and NMR acquisition was less than 5% of independent measurements in six-fold (detection limit: < 1 ppm).

Furthermore this study addressed the use of *in vitro* toxicological profiling like general cyto- and genotoxicity testing (e.g. IEC-6, IEC-18, Caco-2 and INT 407 cells) and the use of eukaryotic and bacterial stress gene assays (i.e. CAT-TOX(L) and PRO-TOX) using transformed human HepG2 and *E. coli* bacteria. Related to this it is recognized that the mucosa of the gastro-intestinal tract is clearly a primary, potential site of action. Toxicity to intestinal cells was not detected in red-ripe non- or transgenic tomatoes using a-GST, LDH leakage, MTT conversion, NR uptake and total cellular protein as *in vitro* endpoints. Green species displayed pronounced toxic and cell-damaging effects, however, no significant

differences were observed between non- and transgenic cultivars. Supplementary investigations with naturally occurring plant compounds (e.g. quercetin, ß-carotene, lycopene, genistein) showed that only the steroidal glycoalkaloid a-tomatine exhibited a rapid toxic effect.

Based on the experience gained it was concluded that application of chemical fingerprint analysis provided information required for the dynamic, analytical establishment of substantial equivalence. When established that the transgenic phenotype is sufficiently different to its appropriate counterpart(s) subsequent analyses might be carried out in order to identify the structure of constituents involved. In case of identical agricultural conditions there were minimal statistically significant differences between isogenic populations. However, variations in the stage of cultivation, location or climate had a significant impact on the overall composition of cultivars. Thus, there is a need for data from multiple locations in order to enable the discrimination between unintended effects of insertional events and those of environmental factors. Moreover, chemical fingerprint studies of digested products are underway to complete the comparative analysis taking into account possible matrix effects.

Genetic modification of the tomatoes did not result in increased cyto- or genotoxicity (i.e. COMET assay). Toxicity of a-tomatine could be easily related to the effects of extracts of green tomatoes containing a known amount of the same compound (30-40 ppm). The in vitro data presented here do not imply that any of the products and tissue extracts tested would have toxic effects in humans or animals but simply constitute baseline data for further studies. The use of molecular endpoints other than cytotoxicity is currently being investigated.

Finally, the development is still at an early stage, but these methods may be an important part in evolving strategies for a tiered approach focusing on comparative analytical and toxicological fingerprint analysis of novel foodstuffs. Approaches that may yield better, more efficient, less costly tests or tests which utilize fewer laboratory animals. Validation of the methodology from the context of its application in prediction of critical changes is necessary.

GENERAL SAFETY ISSUES

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The evaluation of risks to consumer health related to the consumption of transgenic plants or products derived from them must take into account both toxicological and nutritional aspects. This is what is meant by the term "assessment of wholesomeness".

This evaluation, which is based on the concept of substantial equivalence, must take into account knowledge of:

- the characteristics of the host organism;
- the characteristics of the donor organism;
- the genetic modification carried out (techniques used) and the DNA inserted (full description of sequences);
- the characteristics of the genetically modified organism: stability, regulation, sites and levels of expression of the genes introduced.

Special attention must be given to the following points during the toxicological evaluation:

- the potential toxicity of newly expressed proteins;
- the potential toxicity of products resulting from the enzymatic activity of these new proteins (metabolites of pesticides, interference with the hormonal metabolism of the plant, etc.). The objective is to evaluate the potential side effects of the transformation;
- the allergenic potential of the new proteins;
- increased production of natural toxicants;
- potential risks of unexpected, unintended effects (pleiotropic effects);
- risks that a gene introduced may be transferred from the plant to a micro-organism in the intestinal tract (for example, an antibiotic resistance marker gene under the control of a bacterial promoter).

Lastly, the nutritional evaluation must take into account:

- possible variations in the key nutrient content (macro and micro nutrients such as vitamins, trace elements or even certain compounds considered as protectors);
- anti-nutritional compound content (lectins, protease inhibitors, etc.);
- variations in the bioavailability of key nutrients;
- the impact of the appearance of new foods on dietary behaviour and nutritional balance, taking into account specific national and regional dietary patterns.

TOXICOLOGICAL ASPECTS CONCERNING NEW PROTEINS INTRODUCED INTO PLANTS

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At present, the placing on the market of genetically modified plants and derived products designated for consumption in the European Community requires an authorization according to the regulation of Council Directive 90/220/EEC on the Deliberate Release into the Environment of Genetically Modified Organisms. The Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV) is one of the regulatory agencies engaged in the safety evaluation process in Germany.

Focusing on the safety of the newly introduced proteins information is given from the toxicological point of view about the experience with the applications which the BgVV has received up to now for health evaluation. The studies which were usually provided by the applicants (characterization of the expressed proteins, search for homology to known protein toxins and allergens, studies on enzymatic activity and on proteolytic degradation in vitro and toxicity studies in vivo) are described and discussed, referring to problems in the interpretation of results and questions remaining unanswered. Finally, recommendations which are based on the experience with the applications and on the opinion of a Working Group of the Senate Commission on the Evaluation of Food Safety of the Deutsche Forschungsgemeinschaft are presented as a proposal for an adequate testing strategy. In summary, the safety of the introduced proteins should be demonstrated on the basis of a combination of a sufficient number of studies in different fields, which should generally include a short-term feeding study (28 days) in rodents according to the OECD guidelines.

ASSESSMENT OF SUBSTANTIAL EQUIVALENCE BETWEEN GLUFOSINATE TOLERANT MAIZE AND THEIR NON-TRANSGENIC COUNTERPARTS BY PROXIMATE ANALYSES.

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France

Two maize lines have been transformed with the PAT gene by AgrEvo in order to confer tolerance to glufosinate-ammonium. Several commercial inbred lines were reconverted from those primary transformation events.

Three hybrids with the same glufosinate tolerant parent, derived from T.E. T25 were tested in France in 1995, in four locations. In each location each transgenic (GT) hybrid were compared to its non-transgenic counterpart (GS).

In order to assess the substantial equivalence the grain was analyzed with respect to the macronutrient content (starch, cellulose, fat, nitrogen) and micronutrient content (amino acids and fatty acids).

When a high level of isogeny is achieved with both parents, no difference was detected between the transgenic and the non-transgenic version (statistically-significant) but differences could be found between hybrids or between locations. Fatty acids - in particular stearic, oleic, linoleic and linolenic - were revealed as good markers for genetic variations.

Finally it was concluded that it is very important to measure all the interaction: effect of transformation/genetic background/environment.

To achieve this goal it is necessary to compare side-by-side the transgenic and non-transgenic isogenic counterpart, preferably with different pedigrees from the same transformation event, and in several locations

A MODEL SYSTEM TO ASSESS LINKS BETWEEN FOODS AND DEVELOPMENT OF AN AUTOIMMUNE DISEASE, JUVENILE DIABETES.

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Nutrients and other food components can have major effects on the immune system. Although the introduction of new or enhanced levels of allergens to the food supply is a major health concern to a small but highly vulnerable group of consumers, other food-immune system interactions may be important in the development of serious chronic diseases. Approximately 7% of the population of developed nations suffer from some form of autoimmune disease, in which the patient's own immune system reacts against various organs in the body, often causing serious disability or premature death. Juvenile diabetes, one such autoimmune condition, is the most common chronic disease of childhood and incidence is increasing in many parts of the world. There are suggestions that this disease is food-related and this led us to develop a system for evaluating potential diabetes-associated proteins from previously identified plant foods. The system is based on screening protein extracts of suspect foods using Western blotting with sera from control and diabetes-prone rodents or humans. Image analysis of the frequency and intensity of all immunoreactive bands form foods of varying diabetogenicity permitted identification of potential diabetes-related proteins. Future determinations of the benefits and risks of new, modified or functional foods may also consider the use of immunoblotting analyses as they relate to chronic health outcomes in the general population and in vulnerable sub-populations. As new links between foods and immune-based disorders are established, rapid screening techniques for identifying relevant antigens in new foods may assist in risk/benefit analyses.

EDIBLE LUPINS - A CASE STUDY

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The approach followed by the UK Advisory Committee on Novel Foods and Processes (ACNFP) in assessing the food safety of *L. angustifolius* seeds for use in a range of lupin based products was described.

Using the ACNFP's decision tree based approach the information requirements for *L. angustifolius* were identified as:

Intake and extent of use
Nutritional assessment
History of the organism
Toxicological assessment
Human data
Safety information.

Most lupin seeds have a bitter taste due to high levels of alkaloids. However, *L. angustifolius* has a low alkaloid content. The seeds will be consumed as whole seeds, flour, protein concentrates, dietary fibre or fermented products.

Lupin seeds had no adverse effect on growth in rat feeding studies. In addition the ACNFP considered detailed information on mineral content, fatty acid profiles and amino acid profiles.

The bitter varieties of lupins have been consumed for over 3000 years in some countries. *L angustifolius* was first bred commercially in Australia in the 1930s and has an alkaloid of 200-500 mg/kg compared with levels of 5000 - 20 000 mg/kg in bitter varieties.

The toxicological assessment focused on the alkaloid content. Studies assessed included a 90 day feeding study using lupin flour with added quantities of alkaloids and a rat multigeneration study. Mutagenicity studies on the alkaloid extracts were also provided. The ACNFP concluded that the seeds were safe for food use provided the alkaloid content did not exceed 200 mg/kg. The ACNFP noted that lupins can be contaminated by phomopsin mycotoxins, and endorsed the limit of 5 mg/kg set in Australia for phomopsins.

The ACNFP also reviewed information on the allergenic potential of lupins. It was concluded that although lupin flour may induce allergic reactions in susceptible individuals the proportion of the population reacting would be less than that reacting to other legumes such as soya.

In view of the possible allergic reaction the ACNFP ensured that information on the introduction of lupin based foods should be made available to health professionals and allergy support groups.

INFORMATION GENERATED THROUGH THE MARKETING OF FOOD PLANTS

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Most countries require new plant varieties to undergo registration trials to check agronomic qualities, especially yield. The composition of raw food products derived from new varieties is not normally considered for registration purposes, except where specific qualities are important for end users (e.g., bread making quality of wheat flour). This reflects the long history of safe use of our major crop species. Within the last few years there have been trends toward more diversification of the market for commodity food crops. End-users have identified unique nutritional qualities that justify the cost of segregation (e.g., high oil maize). The need to confirm the quality and value of such identity preserved products is causing grain handlers to develop methodologies that rapidly and economically analyze the gross composition of grains and oilseeds. There is also increased interest in selecting and developing products for specific end users (e.g., high methionine grain for poultry feeding) which has caused suppliers of raw materials to the compounded feed and processed food industries to undertake more comprehensive analysis of their products (e.g., amino acid and fatty acid profiling). As a result, developers are beginning to generate databases that reflect the composition of our major agricultural commodities. In some instances, developers have initiated food safety evaluations to provide potential customers with assurances as to the safety of products derived from new plant varieties with altered characteristics. In such cases, the approach has generally been to use compositional analysis of key nutrients and anti-nutrients to show that these products are substantially equivalent to raw food materials currently in commerce.

DEVELOPMENT AND USE OF IN VITRO INTESTINAL TRACT MODEL FOR SAFETY EVALUATION OF NOVEL FOODS.

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In order to evaluate the biological safety of genetic modified foods it is essential to have adequate models available. These could be used as early warning systems which may reduce the use of test animals for nutritional studies and gene transfer studies. TNO has developed a gastro-intestinal model (TIM), simulating to a high degree the physiology of the stomach and the intestine of monogastric animals and man. This was achieved by simulating the successive kinetic physiological events such as temperature, pH, saliva, gastric and intestinal secretions (electrolytes, enzymes, co-factors, bile, pancreatic juice), gastric and intestinal mixing and transporting by peristaltic movements plus absorption of water and small molecules (released and digested food compounds). TIM is currently being validated by a team of researchers from different disciplines for new research possibilities, including digestion of proteins, carbohydrates and fats, absorption and bio-availability of digested products, the survival and interactions of ingested bacteria. In addition TIM contains a large intestine including a dense and complex anaerobic human microflora. Short term fermentation experiments have been performed successfully in this system. The gas and acid production can be continuously monitored, whilst the composition of the microflora, the produced fatty acids and gas mixtures can be analyzed.

TIM is an alternative for human and animal experiments, having a high extrapolative value.

This because TIM was successfully validated in comparison to in vivo experiments with human volunteers and fistulated pigs and calves.

The application potential of TIM has been demonstrated. In particular an insight was provided in the validation of the model for conjugal transfer of DNA. In addition, the effect on the integrity of free DNA in the different compartments of TIM was presented. The integrity of the DNA is particularly affected in the stomach and small intestine. The half-time of intact DNA was less than 10 seconds. In the large intestine the situation was different. The half-time of intact DNA was approximately 4 to 6 minutes. However, no transformants using a broad host-range plasmid could not be detected in the large intestine so far. The sensitivity of detection using a PCR system was limited to 1 plasmid containing cel per 10E9 colony forming units of the colon-flora after introducing plasmid containing E. coli cells.

In conclusion, TIM is well equipped for the nutritional evaluation of novel foods. With respect to the horizontal transfer of genes, which is also essential for the assessment of food safety, additional efforts are required.

LESSONS LEARNED IN THE EVALUATION OF NOVEL FOOD: THE EXPERIENCE OF THE UK ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

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Over a period of about nine years the ACNFP has considered the safety evaluation of a great variety of novel foods and processes. The novel foods have included GM organisms (microorganisms and higher plants) and enzymes and products derived from these, as well as novel food sources, fat-replacers and novel fats and products of novel processing technology.

In dealing with this variety of novel foods, the level of testing required has been dependent on the nature of the 'novelty,' the extent and circumstances of human exposure and the availability of human data. On the basis of the accumulated experience, a decision three approach was formulated and refined, and a brief description of this was presented.

A number of generic issues also have emerged in the course of evaluations and particular attention was paid to the use of antibiotic resistance markers, particularly in microorganisms which are to be consumed as such. Guidelines on taste testing and nutritional assessment of novel foods also have been formulated.

The importance of having appropriate human data, particularly to address the issues of tolerance or idiosyncratic intolerance has been emphasized.

Although the ACNFP decision has now been superseded by EU guidelines, the general principles emerging remain valid and similar approaches to evaluation of novel foods will be adopted.

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