

**GM FOOD CROPS AND APPLICATION OF
SUBSTANTIAL EQUIVALENCE
IN THE EUROPEAN UNION**

*Schenkelaars Biotechnology Consultancy,
The Netherlands, June 2001*

CONTENT

1. Executive summary	page 3
2. Background	page 7
3. Historical analysis of the concept of substantial equivalence	page 9
4. Analysis of the concept of substantial equivalence in Europe	page 13
5. Analysis of case studies of substantial equivalence assessments	page 16
Technical Annex I History of the concept of substantial equivalence	page 27
Technical Annex II The concept of substantial equivalence and European legislation	page 35
Technical Annex III Case studies of substantial equivalence assessments in Europe	page 42

EXECUTIVE SUMMARY

History of the concept of substantial equivalence

- The assumption that organisms modified by rDNA techniques do not pose unique hazards compared to organisms modified by traditional means forms the conceptual cornerstone of OECD guidelines for safety in biotechnology. This assumption has however been challenged in scientific literature and empirical data are lacking to validate the simple linear model of 'precise' genetic engineering.
- In 1993, based on its conceptual cornerstone for safety in biotechnology, OECD introduced the concept of substantial equivalence as a guiding principle in the food safety assessment to detect intended and unintended differences between a GM food (component) and its traditional counterpart. This approach has been developed, because in contrast to many compounds such as pesticides, pharmaceuticals, industrial chemicals and food additives, whole (GM) foods are complex mixtures of compounds characterised by a wide variation in composition and nutritional values. Their safety is therefore difficult to assess by conventional toxicological approaches involving for example animal feeding experiments.
- At the end of the 1990s the concept and its application in regulatory decision-making started to attract considerable criticism, as several authors viewed it as an excuse for not requiring toxicological tests. In 2000 expert meetings convened by OECD and FAO/WHO reviewed the application of the concept of substantial equivalence. These meetings resulted in endorsement of the concept in a general sense. However, reports presented at these meetings indicated that there had been a lack of consistency from case to case in the data provided, even within the same crop species. This led to more sophisticated discussions on data requirements to determine substantial equivalence of a GM food crop or ingredients derived thereof, as well as on the 'traditional counterpart' or 'selected comparator' to be used for comparison and on methods to generate statistically valid data. The expert meetings further recommended developing a database containing baseline concentrations of plant compounds of potential nutritional or toxicological concerns and knowledge on how concentrations of these compounds may vary depending on the genetic background of the plants and environmental conditions.
- According to the OECD and FAO/WHO expert meetings in 2000, present approaches to detect unintended differences between a GM food crop or component and a 'selected comparator' due to genetic modification are based on chemical analysis targeted at specific (known) compounds. Further development and validation of profiling techniques based on genomics, proteomics and metabolomics may increase the potential to detect unintended differences.

European legislation and substantial equivalence

- Until 2000, within the framework of Regulation 258/97, the concept of substantial equivalence has triggered a series of regulatory decisions by the European

Commission and national competent authorities whether a 'light' notification procedure or a 'heavy' authorisation procedure had to be followed.

- When Regulation 258/97 came into force in January 1997, an operational definition of the concept of substantial equivalence was not available. Nonetheless, case by case, several GM plants and/or ingredients thereof have been determined as substantially equivalent (except for the modified trait) and notified for food use in the EU. However, according to literature, relevant data about inherent plant toxins and anti-nutrients were often missing or showed significant differences. In addition, data for comparisons showed inconsistency from case to case, even within the same plant species.
- An operational definition of substantial equivalence is still lacking. There is for example no minimum list of macro- and micro-nutrients, inherent plant toxins, anti-nutrients, secondary plant metabolites and allergens known to be associated with a crop species, which should be analysed, for the determination of a GM food crop as substantially equivalent. Further, discussions on valid methods to generate compositional data of a GM food crop and its 'control' from field trials and on their statistically analysis have not yet been completed by EC scientific committees and competent authorities of EU member states.

Transparency of regulatory decision-making in the EU

- In practice mainly the UK, Germany and The Netherlands have received requests for notification or authorisation of GM food crops. Opinions of EC scientific committees and assessment reports by the competent authorities of the United Kingdom and The Netherlands on the safety evaluation of GM food crops, including a determination of their substantial equivalence, are publicly disclosed and relatively easily accessible through the Internet. The data submitted by applicants are also made publicly available at governmental libraries in these countries. In Germany, however, the competent authority does not have a mandate to publicly disclose the data submitted and its assessment reports under Regulation 258/97. Its substantial equivalence assessments were based on information submitted for commercial releases within the framework of Directive 90/220. In Germany these 90/220 application files are not made publicly available, whereas at a governmental library in The Netherlands these files are publicly accessible.

Findings of the case studies on GM rape

- Initial assessments by the German, respectively UK competent authority determined refined oil from Liberator Phoe6/Ac, Falcon GS40/90, MS8xRF3 and Topas 19/2 as substantially equivalent. The UK authority requested the applicant to monitor the seed composition and fatty acid profile of the oil of Topas 19/2 over time, as there was little experience in predicting the effect of genetic drift on the metabolism of any plant, whether GM or conventionally bred. In all these cases, the EC Scientific Committee on Plants also determined (refined oil of) these GM rape plants as substantially equivalent (except for the modified trait).

- However, in all cases differences in composition of the oil and/or the meal (for feed use) between the GM-plant and its non-GM control have been observed by member states. Further, from case to case, there has been a lack of consistency in compositional data submitted on the content of macro- and micronutrients, minerals, vitamins, inherent plant toxins and anti-nutrients. The content of sinapine, an anti-nutrient of rape, has not been determined in all cases. In addition, the design of the field trials, the number of locations and seasons, and the choice of the 'comparator' have considerably differed from case to case. It is hardly plausible that the compositional data have been analysed in a statistically sound way.

Findings of the case studies on GM maize

- The initial assessments for notification of Bt11 silage maize, T25 and MON810 by the UK authority determined that these GM maize plants did not differ in composition from their non-GM controls. As there was little experience in predicting the effect of genetic drift on the metabolism of any line of plants, whether GM or non-GM, the UK authorities asked the applicant to monitor the seed composition and the fatty acid and amino acid profile of the GM maize over time. In 1998 the EC Scientific Committee on Plants determined Bt11 (silage) maize as substantially equivalent, whereas in 2000 this committee did not explicitly reach the conclusion that it should be viewed as substantially equivalent. T25 and MON810 were both determined to be substantially equivalent (except for the modified trait) by the EC Scientific Committee on Plants.
- However, in all these cases of notifications differences in the composition of the GM maize plant and its non-GM control have been observed. Further, from case to case, there has been a lack of consistency in compositional data submitted on the content of macro- and micro-nutrients, minerals, vitamins, inherent plant toxins and anti-nutrients. For example, the content of trypsin inhibitor, an anti-nutrient in maize, has not been determined in all cases. In addition, the design of the field trials, the number of locations and seasons, and the choice of the 'comparator' have considerably differed from case to case. It is hardly plausible that the compositional data have been analysed in a statistically sound way.
- The requests for authorisation of Bt11 *sweet* maize and GA21 have been submitted to the Dutch authorities, which treated the data submitted in a rather critical way. Applicants were urged to provide additional data on the content of five secondary metabolites in the GM maize plants compared to their non-GM controls to underpin the degree of substantial equivalence. Such data were provided, but it did not lead unambiguously to a determination of (the degree of) substantial equivalence of GA21. The Dutch authorities noted that applicants would be helped by concrete guidance concerning the number of samples, locations and years, which would be needed for the quantitative analyses.
- In both these cases of a 'heavy' authorisation procedure, several member states raised critical questions on the assessment by the Dutch authorities. In the case of Bt11 *sweet* maize the applicant has provided a response to these questions. In the case of GA21 it is not clear whether the applicant has provided additional information. In both cases it is unclear how these questions and responses

impact regulatory decision-making by the European Commission and national authorities of EU member states, as the EC Scientific Committee on Plants has determined Bt11 (sweet) maize as well as GA21 maize as substantially equivalent to their non-GM controls.

- The request for authorisation of crosses of T25 and MON810 has been submitted to the Dutch authorities, which have not yet completed their initial assessment. The EC Scientific Committee on Plants concluded that T25xMON810 hybrids are substantially equivalent to T25 and MON810 and non-GM maize.

Findings of the case study on GM tomato TGT7F

- The applicant sought a (full) safety evaluation of processed products of GM tomato TGT7F. The UK authority concluded that no nutritional and toxicological differences existed between the GM tomato and its control. The UK authority did not explicitly establish the GM tomato as substantially equivalent, whereas the EC Scientific Committee on Plants and the EC Scientific Committee on Food both concluded that the GM tomato is substantially equivalent.
- Compositional data have been obtained from trials during one year. It is not clear whether these data have been analysed in a statistically sound way. Further, data of the GM tomato and its control regarding several inherent tomato toxins, such as tomatidine, aglycone of tomatine, saponines, coumarins, protease-inhibitor and oxalate, were not provided.

General conclusions

- There are methodological limitations for obtaining meaningful information from conventional toxicological studies on whole (GM) foods. Irrespective of the issue whether genetic modification of food crops involves unique risks compared to conventional breeding, the concept of substantial equivalence could be a guiding principle to detect intended and unintended differences between a GM food crop and its non-GM control to address these limitations. Differences detected should then be focus of further nutritional, toxicological and immunological evaluation.
- In the EU the concept of substantial equivalence urgently requires an appropriate, operational definition, in particular for deciding whether a 'light' notification procedure could be followed.
- An operational definition of substantial equivalence should include detailed protocols on the design of the field trials for collecting compositional data of a GM food crop and its non-GM reference. It should also include a minimum list of macro- and micro-nutrients, anti-nutrients, inherent plant toxins, secondary metabolites and allergens to be analysed for each crop species. It should further foresee in validated techniques to establish the content of these compounds in the (GM) plants and common methods to statistically analyse the data. Differences in the composition of a GM food crop and its non-GM reference, whether intended or unintended, should then be subject to a further safety assessment.

1. BACKGROUND

1.1 Introduction

In the EU Regulation 258/97 on Novel Foods and Novel Ingredients regulates the food use of (ingredients of) genetically modified (GM) plants. This regulation provides for a simplified procedure for foods derived of genetically modified organisms (GMOs) but no longer containing GMOs which are 'substantially equivalent' to existing foods. In such cases the companies only have to notify to the Commission when placing the novel food or novel ingredient on the market. The product can then be marketed in the entire EU. If a GM plant (or ingredients derived thereof) is not determined as 'substantial equivalent', the regulation foresees a full authorisation procedure. Hence, the concept of 'substantial equivalence' plays a decisive role in regulatory decision-making on the food use of (ingredients of) GM plants in the EU.

Moreover, Regulation 258/97 stipulates that no later than five years from the date of entry into force and in the light of the experience gained, the Commission shall forward to the European Parliament and to the Council a report on its implementation. The date of entry was 27 January 1997, which implies that the Commission should forward this report at the latest on 27 January 2002.

Further, the Dutch Foundation 'Consument en Biotechnologie' is involved in a project to actively involve consumer organisations in the further development of regulatory policies on genetically modified foods and their safety evaluation in the European Union. The project is an initiative of Consumentenbond, the Dutch Consumers Union, and has received a grant from the European Commission Directorate General Health and Consumer Protection. In the fall of 2001 a workshop will be convened to have an exchange of views on the implementation of Regulation 258/97 between food officers of national consumer organisations and the European consumer organisation BEUC, scientists and representatives of national competent authorities and the European Commission. In addition, BEUC, participating in ENTRANSFOOD, a European research project on the food safety assessment of genetically modified food crops, will be enabled to provide adequate input into this research project.

Within this context the Dutch Foundation 'Consument en Biotechnologie' commissioned Schenkelaars Biotechnology Consultancy to prepare an analysis of international and European regulatory discussions on the concept of substantial equivalence. This analysis should also include a set of case studies on how this concept has been applied in notifications and authorisations of (ingredients of) several GM foods crops the EU. The analysis should thereby mainly focus on notifications, as in these cases determination of a GM plant (or ingredient thereof) as substantially equivalent triggers the regulatory decision that it can be placed on the market.

1.2 Material and methods

The historical analysis of regulatory and scientific discussions on the concept of substantial equivalence has been based on reports of expert meetings convened from 1990 to 2000 by the Organisation for Economic Co-operation and Development

(OECD) and the United Nations Food and Agriculture (FAO) and World Health Organisation (WHO). Reports of expert meetings held in the United States in 1999 and Canada in 2000 are also reviewed. This analysis can be found Chapter 2 of this report. Short summaries of the aforementioned documents can be found in Technical Annex I.

The analysis of the concept of substantial equivalence in relation to European legislation has been based on a review of the European Regulation 258/97, opinions delivered by several scientific committees of the Commission, a paper by the United Kingdom Food Standards Agency, and a review article published by a scientific journal. This analysis can be found in the Chapter 3. Technical Annex II contains short summaries of these documents.

The case studies have been based on various documents obtained from the websites of the European Commission and of national competent authorities of Germany, The Netherlands and the United Kingdom. Several of these documents have been accessed at the libraries of the Dutch Ministry of Public Health, respectively the Ministry of Environment. Additional information has been obtained through personal communication with Mrs Van de Wiel of the Dutch advisory body, with Mrs Schauzu of the German competent authority and with Mr Ball of the United Kingdom advisory body. The analysis of the case studies can be found in Chapter 4. Technical Annex III contains detailed accounts of the findings in each case.

The full references, on which this report has been based, can only be found in the technical annexes.

1.3 Scope of the study

The focus of this study is on the ways the concept of substantial equivalence has been discussed by various international and European bodies and on its application by the European Commission and national competent authorities.

Approaches applied by applicants and regulatory authorities to assess potential toxicological and immunological impacts of newly introduced DNA or protein(s) in a GM food crop are beyond the scope of this study. Main reason is that there seems to be general scientific and regulatory consensus that newly introduced DNA or protein(s) are not to be considered as substantially equivalent and therefore require particular attention *per se* in the food safety assessment of (ingredients of) GM food crops.

Hence, this study does *not* evaluate the full food safety assessments of GM food crops as the European Commission and national competent authorities have carried these out.

2. HISTORICAL ANALYSIS OF THE CONCEPT OF SUBSTANTIAL EQUIVALENCE

2.1 Introduction

In the beginning of the 1970s the advent of recombinant DNA (rDNA) techniques, which enabled splicing DNA molecules and reintroducing them into living organisms, almost immediately prompted the first discussions among scientists on whether the use of these rDNA techniques would go associated with unique hazards. It also led to the first discussions among governmental policymakers whether application of rDNA techniques should be regulated. At that time application of these rDNA techniques was limited to laboratories and the first safety guidelines were therefore developed for the use of rDNA organisms in confinement. About ten years later these techniques were also increasingly applied to develop novel organisms for experiments and usage outside laboratories. In 1983 this led the Organisation for Economic Co-operation and Development (OECD) to develop in general safety guidelines for the use genetically modified organisms (GMOs) in industry, agriculture and the environment, which were published in 1987. An important principle was to focus safety concerns on whether organisms modified by rDNA techniques pose an 'incremental' risk to organisms modified by conventional means. Although application of rDNA techniques might result in organisms with a combination of traits not observed in nature, genetic changes from rDNA techniques would often have a greater predictability compared to traditional means because of the greater precision of rDNA techniques. The expectation therefore was that any risk associated with application of GMOs might be assessed in generally the same way as those associated with non-GMOs.

This conceptual cornerstone of the OECD safety guidelines, upon which regulations for safety in biotechnology of most industrialised countries have been based, has however not been without any scientific controversy. In scientific literature several authors have argued that unique hazards do exist in the movement of genes between unrelated organisms by applying rDNA techniques. Also a few scientists of the US Food and Drug Administration (FDA) have cautioned this agency in the beginning of the 1990s that foods produced through rDNA technology entail different risks than do their conventional counterparts but their view has been disregarded. Moreover, two expert committees, one convened by the US National Research Council in 1999 and the other one convened by the Royal Society of Canada in 2000, concluded that empirical data were lacking to validate the simple linear model of 'precise' genetic engineering.

2.2 Emergence of the concept of substantial equivalence in 1993

Three years after the publication of general safety guidelines for the use of GMOs, in 1990, the OECD established a working group for developing scientific principles on the safe use of foods derived by modern biotechnology. In 1993 this working group recommended to base the scientific approach to the safety evaluation of a new food or food component on the comparison with traditional foods that have a safe history of use. This approach was in turn based on the concept of substantial equivalence,

which articulated procedures used in the past, albeit intuitively, for accepting new foods. Use of this approach should lead to reasonable certainty that no harm will result from the intended uses under anticipated conditions of consumption, as in the working group's view foods derived by modern biotechnology were inherently not less safe than their origin counterparts. A fundamental change in the safety assessment of such foods was therefore not considered as necessary, nor should a different standard of safety be required. The principle was set out that if a modified food or food component would be determined to be substantially equivalent to an existing food, it could be treated in the same manner. No additional safety concerns would be expected. However, when a food or food component would not be determined to be substantially equivalent, the identified differences should be the focus of further evaluations. A number of theoretical case studies illustrated how 'substantial equivalence' might be applied.

An important rationale for developing this approach to the safety assessment of GM foods was that in contrast to many compounds such as pesticides, pharmaceuticals, industrial chemicals and food additives, whole (GM) foods are complex mixtures of compounds characterised by a wide variation in composition and nutritional values. To the opinion of the OECD working group, there were serious practical and methodological difficulties of obtaining meaningful information from conventional toxicology studies on the safety of whole foods. These difficulties had for example also become apparent when animal feeding studies had been used to assess the safety of irradiated foods.

In 1996 the United Nations Food and Agriculture Organisation (FAO) and World Health Organisation (WHO) also convened an expert consultation to address approaches to assess the safety of GM foods. With reference to the work by OECD, this consultation recommended that the concept of substantial equivalence was an important component in the safety assessment of foods and food ingredients derived from GM plants.

2.3 Criticism on 'substantial equivalence' in 1999

In October 1999 the scientific journal *Nature* published a critical view on the concept of substantial equivalence, which attracted wide attention. The authors argued that the degree of difference between a natural food and its GM alternative before its 'substance' ceases to be acceptably 'equivalent' had not been defined, nor had legislators agreed an exact definition. The authors felt that the vagueness of the concept might make it useful to industry but unacceptable to the consumer. The concept was considered as inherently anti-scientific because it was created primarily to provide an excuse for not requiring toxicological tests. Although the authors acknowledged that biochemical and toxicological tests and their interpretation are notoriously problematic and contested, and slow and expensive, they could provide information vital to consumer protection. Companies should have been required to conduct a range of toxicological tests to set 'acceptable daily intakes'.

The OECD however argued that such classical toxicological tests might also well reveal that traditional foods often contain natural toxicants and/or anti-nutritional substances with undesirable effects in animal experiments. It would therefore be unreasonable and unscientific to expect an absolute standard of safety for GM foods,

which could be demonstrated through classical toxicological tests. The important starting point in the safety assessment should therefore be a comparison between the new GM food and its traditional counterpart. However, application of substantial equivalence was not in itself to be viewed as a substitute for a safety assessment. The concept had been conceived as a guiding principle, which was intended to be a tool for those engaged in food safety assessments of GM foods to detect intended and unintended differences between a GM food or food component and its 'traditional counterpart' or 'selected comparator'. The OECD also pointed out that there was work underway to identify the critical nutrients and toxicants found in major crops, which would be an important focus of a demonstration of substantial equivalence.

2.4 International reviews of 'substantial equivalence' in 2000

Since 1995 regulators from countries in North America, Europe and Asia had gained practical experience in applying the concept of substantial equivalence but there had been no universal consensus on its application. In 2000 a series of expert meetings convened by OECD, respectively by FAO/WHO reviewed the concept, its limitations and its role in regulatory decision-making. In a general sense all these expert meetings endorsed the concept of substantial equivalence as a tool in the safety assessment of GM foods and GM food ingredients.

Reports presented at these meetings however indicated that the comparison principle of substantial equivalence had normally been used in regulatory decision-making but there had been a lack of consistency from case to case in the data provided, even within the same crop species. Consequently, discussions on data requirements became more sophisticated. For example, when determining substantial equivalence of a GM food crop and/or its ingredients, one of the expert meetings recommended that the 'selected comparator' should be ideally the near isogenic parental lines grown under identical conditions. It further recommended developing guidelines for appropriate design of field trials with GM food crops and guidelines for key substances, nutrients and data needed for assessment of substantial equivalence. It also recommended that data for comparison should be obtained using validated methods and analysed using appropriate statistical techniques.

Another aspect addressed by these meetings was a need for a data base containing baseline concentrations of plant compounds of potential nutritional or toxicological concerns and knowledge on how concentrations of these compounds may vary depending on the genetic background of the plants and environmental conditions. In addition, these expert meetings indicated that present approaches to assess possible unintended effects are based on chemical analyses targeted at specific (known) compounds. In order to increase the probability of detecting unintended effects, a further development and validation of profiling techniques based on genomics, proteomics and metabolomics was generally recommended to detect differences in a more extensive way than targeted chemical analysis.

2.5 Main findings

- The assumption that organisms modified by rDNA techniques do not pose unique hazards compared to organisms modified by traditional means forms the conceptual cornerstone of OECD guidelines for safety in biotechnology. This assumption has however been challenged in scientific literature and empirical data are lacking to validate the simple linear model of 'precise' genetic engineering.
- In 1993, based on its conceptual cornerstone for safety in biotechnology, OECD introduced the concept of substantial equivalence as a guiding principle in the food safety assessment to detect intended and unintended differences between a GM food (component) and its traditional counterpart. This approach has been developed, because in contrast to many compounds such as pesticides, pharmaceuticals, industrial chemicals and food additives, whole (GM) foods are complex mixtures of compounds characterised by a wide variation in composition and nutritional values. Their safety is therefore difficult to assess by conventional toxicological approaches involving for example animal feeding experiments.
- At the end of the 1990s the concept and its application in regulatory decision-making started to attract considerable criticism, as several authors viewed it as an excuse for not requiring toxicological tests. In 2000 expert meetings convened by OECD and FAO/WHO reviewed the application of the concept of substantial equivalence. These meetings resulted in endorsement of the concept in a general sense. However, reports presented at these meetings indicated that there had been a lack of consistency from case to case in the data provided, even within the same crop species. This led to more sophisticated discussions on data requirements to determine substantial equivalence of a GM food crop or ingredients derived thereof, as well as on the 'traditional counterpart' or 'selected comparator' to be used for comparison and on methods to generate statistically valid data. The expert meetings further recommended developing a database containing baseline concentrations of plant compounds of potential nutritional or toxicological concerns and knowledge on how concentrations of these compounds may vary depending on the genetic background of the plants and environmental conditions.
- According to the OECD and FAO/WHO expert meetings in 2000, present approaches to detect unintended differences between a GM food crop or component and a 'selected comparator' due to genetic modification are based on chemical analysis targeted at specific (known) compounds. Further development and validation of profiling techniques based on genomics, proteomics and metabolomics may increase the potential to detect unintended differences.

3. THE CONCEPT OF SUBSTANTIAL EQUIVALENCE AND EUROPEAN LEGISLATION

3.1 European Community Regulation on Novel Foods

On 25 January 1997 Regulation 258/97 on Novel Foods and Novel Food Ingredients came into force in the EU. Food or food components derived of GM plants also fall under this regulation, which foresees a simplified notification procedure if such GM food or food component is determined as 'substantially equivalent' to an existing food with respect to composition, nutritional value and metabolism. In other cases a full authorisation procedure must be followed.

On 29 July 1997 the Scientific Committee for Food (SCF) of the European Commission published its recommendations concerning the scientific aspects and the information necessary to support an application of a novel food or novel food ingredient. With reference to recommendations by OECD and FAO/WHO the SCF reminded that determination of substantial equivalence is not a safety or nutritional assessment in itself but an approach to compare a novel food with its conventional counterpart. Analytical studies were viewed of crucial importance for the establishment of substantial equivalence as well as for nutritional and toxicological assessments. Methods had to be standardised and validated. SCF further recommended to focus investigations especially on the content of critical macro- and micro-nutrients, any critical toxicants and anti-nutritional factors which might be either inherently present or due to the genetic modification.

On 28 December 1998 the Scientific Committee on Plants (SCP) of the European Commission published a guidance document to facilitate applicants in the preparation of GM plant dossier. One of its recommendations was that data should be obtained from a valid comparison of GM plants and a conventional, preferably isogenic line, based on samples from at least two seasons from a number and variety of geographical locations and accompanied by an appropriate statistical analysis. In May 1999 the scientific advisory body to the UK regulatory authority also presented a paper with considerations for statistically valid data to the European Commission to promote discussion.

In October 2000 the Scientific Steering Committee of the European Commission endorsed substantial equivalence as a starting point to guide the safety evaluation. It noted that several scientific committees had used the concept on a case by case base and therefore needed an operational definition of the concept. Further, the routine analytical procedures in use also needed to be standardised in terms of sampling and extraction procedures, validation of profiling methods and in bio-informatics.

Finally, in 2001 the Scientific Committee on Plants (SCP) began drafting a guidance document to facilitate applicants in the preparation of GM plant dossiers for an authorisation of a GM plant release (Directive 90/200 and Directive 2001/18) and subsequently for a GM food (Regulation 258/97) (and/or feed) authorisation. The opinion would fall under the remit of three scientific committees: Scientific Committee on Food (SCF), Scientific Committee on Animal Nutrition (SCAN) and SCP. The

guidance document should in principle be adopted by the Scientific Steering Committee (SSC). The document by SCP would provide guidance for the molecular characterisation of the gene construct, for the establishment of substantial equivalence and for performing the safety assessment, the nutritional assessment and the environmental assessment.

3.2 Substantial equivalence assessments in the EU

Since Regulation 258/97 came into force in January 1997, an operational definition of the concept of substantial equivalence for decision-making has been and still is lacking. Nonetheless, since 1995 several GM plants and/or ingredients thereof have been determined as substantially equivalent and notified for food use in the EU. In several cases of authorisation the concept of substantial equivalence also played an important role in their safety assessment.

3.3 Review of substantial equivalence assessments

In 2000 a scientific review article (Kovak 2000), based on an analysis of data submitted for notification of the food use of several GM plants and/or ingredients thereof in the US and EU until 1998, generally supported the use of substantial equivalence. Nonetheless, this review article revealed that relevant data about inherent plant toxins and anti-nutrients were often missing or showed significant differences. Data for comparisons showed inconsistency from case to case. It was therefore recommended to draft a minimum list of macro- and micro-nutrients, inherent plant toxins, anti-nutrients, secondary plant metabolites and allergens known to be associated with a crop species. In one case significant differences between a GM oilseed rape plant and its control in the values for an inherent plant toxin had been observed but its content varied more between the different locations than between different GM and non-GM oilseed rape lines at a given location. According to the applicant, it was generally accepted that commercial food derived of plants exhibit considerable variability in its composition. In this case the differences observed were more the result of the interaction of the genotype with the environment rather than the result of the insertion of specific genes into the plant genome. Since this might not be scientifically justified, the authors raised question such as: How much is the accepted range of variability? What are the regulations for variations above the statistical limits? And what variations might be dangerous? It was also pointed out that literature data sometime show wide variations in typical plant components and may not be directly comparable due to differences in analytical methods or sample preparations. Further, literature data are often relatively old and may not encompass the compositional variables of modern crop varieties. Another problem for obtaining appropriate data observed by the authors was that it is technically not always possible to use authentic isogenic lines to compare the GM lines with.

Another scientific article (Noteborn 2000) described a recently developed chemical fingerprinting methodology that could contribute to the demonstration of substantial equivalence by its ability to compare compositional changes with regard to a couple of hundred low-molecular compounds between a GM food crop and related non-GM reference lines. The authors also proposed a hierarchical approach by comparing the

chemical fingerprints of the GM plant to those of: 1) isogenic parental or closely related lines bred at identical and multiple sites; 2) extended ranges of commercial varieties of that plant; and 3) downstream processing effects. Such an approach was recommended to assess the likelihood that the statistical differences in a GM crop plant may be false positives due to chance alone, or arose from natural genetic and/or physiological variations.

3.4 Main findings

- Until 2000, within the framework of Regulation 258/97, the concept of substantial equivalence has triggered a series of regulatory decisions by the European Commission and national competent authorities whether a 'light' notification procedure or a 'heavy' authorisation procedure had to be followed.
- When Regulation 258/97 came into force in January 1997, an operational definition of the concept of substantial equivalence was not available. Nonetheless, case by case, several GM plants and/or ingredients thereof have been determined as substantially equivalent (except for the modified trait) and notified for food use in the EU. In several cases of authorisation the concept of substantial equivalence also played an important role in their safety assessment. However, according to literature, relevant data about inherent plant toxins and anti-nutrients were often missing or showed significant differences. In addition, data for comparisons showed inconsistency from case to case, even within the same plant species.
- An operational definition of substantial equivalence is still lacking. There is for example no minimum list of macro- and micro-nutrients, inherent plant toxins, anti-nutrients, secondary plant metabolites and allergens known to be associated with a crop species, which should be analysed, for the determination of a GM food crop as substantially equivalent. Further, discussions on valid methods to generate compositional data of a GM food crop and its 'control' from field trials and on their statistical analysis have not yet been completed by EC scientific committees and competent authorities of EU member states.
- Recent advances in chemical fingerprinting of a couple of hundred low-molecular compounds in complex plant matrices could further contribute to detect unintended changes in the composition of a GM food crop compared to its non-GM reference lines.

4. CASE STUDIES OF SUBSTANTIAL EQUIVALENCE ASSESSMENTS IN EUROPE

4.1 Collection of regulatory information and data submitted

To analyse the application of substantial equivalence in notification and authorisation procedures in the EU, detailed case studies have been prepared on:

- Notification of refined oil of GM rape Liberator Phoe6/Ac.
- Notification of refined oil of GM rape Falcon GS40/90.
- Notification of refined oil of GM rape MS8xRF3.
- Notification of refined oil of GM rape Topas 19/2.

- Notification of processed products of GM Bt11 silage maize.
- Request for authorisation of processed products of GM Bt11 sweet maize.
- Request for authorisation of processed products of GM maize GA21.
- Notification of processed products of GM T25 maize.
- Notification of processed products of GM MON810 maize.
- Request for authorisation of processed products of conventional crosses of GM maize T25 and GM maize MON810.

- Authorisation of processed products of GM tomato TGT7F in the UK.
- Request for authorisation of processed products of GM tomato TGT7F in the EU.

The findings described below are based on the detailed accounts of each case presented in Technical Annex III. The information for these accounts has been obtained in several manners.

Documents with opinions on substantial equivalence delivered by the Scientific Committee for Foods and the Scientific Committee on Plants of the European Commission were accessible at the website of the European Commission. These documents also contained a short summary of the data submitted. The original data sets, on which the substantial equivalence assessments have been based, were however not publicly available through the Internet.

Initial assessment reports on substantial equivalence of (ingredients of) GM plants were available at the website of the competent authority of the United Kingdom, or have been obtained through correspondence. The data sets submitted can in principle be accessed at the British library.

The German authority indicated that it had no mandate to make its initial assessment reports on notifications of (ingredients of) GM plants made publicly available. It further indicated that no separate notification files for the food use of refined oil of these three GM oilseed rape plants had been submitted. Its substantial equivalence assessments were based on information submitted in the application of Liberator Phoe6/Ac, Falcon GS40/90 and MS8xRF3 for commercial release within the framework of Directive 90/220. These application files are neither publicly disclosed by the German authorities.

In the Netherlands, similar to the UK, initial assessment reports by the Dutch authorities are made publicly available at the website of the scientific advisory body to the Ministry of Public Health. Further, upon request, the data sets submitted and correspondence with the European Commission, national authorities of other EU member states and the applicants are also made available. Noteworthy, the Dutch authorities also disclose, upon request, the initial assessment reports by the German authorities on Liberator Phoe6/Ac, Falcon GS40/90 and MS8xRF3.

Moreover, the Ministry of Environment, the Dutch authority for Directive 90/220 on the deliberate release into the environment of GMOs, has made all application files for experimental and commercial releases of GMOs publicly accessible at its library with relatively little information kept confidential. Information on the data submitted in the case of Liberator Phoe6/Ac, Falcon GS40/90 and MS8xRF3 could therefore be collected from these application files.

It should also be noted that collecting the information for the case studies on substantial equivalence assessments of (ingredients of) GM food crops by various scientific committees of the European Commission and EU member states required substantial efforts. It should also be noted that original data sets submitted to determine substantial equivalence have until now not been routinely exchanged between the member state, which drafted the initial assessment report, and other member states and the European Commission. In addition, opinions of scientific committees of the European Commission and initial assessment reports by national authorities of EU member states, which could be accessed, all contained different kinds of summaries of information and data, on which regulatory decisions have been based. There is however a lack of transparency about the way these authorities have summarised the information and data submitted in each case.

In summary, opinions of EC scientific committees and assessment reports by the competent authorities of the United Kingdom and The Netherlands on the safety evaluation of GM food crops, including a determination of their substantial equivalence, are publicly disclosed and relatively easily accessible through the Internet. The data submitted by applicants are also made publicly available at governmental libraries in these countries. In Germany, however, the competent authority does not have a mandate to publicly disclose the data submitted and its assessment reports under Regulation 258/97. It turned out that its substantial equivalence assessments were based on information submitted in the application of Liberator Phoe6/Ac, Falcon GS40/90 and MS8xRF3 for commercial release within the framework of Directive 90/220. In Germany these application files are not made publicly available, whereas at a governmental library in The Netherlands these files are publicly accessible.

4.2 Analysis of case studies on GM rape

4.2.1 Liberator Phoe6/Ac (AgrEvo/Aventis)

The application file, which has been submitted under Directive 90/220 in Germany in 1998, indicated that the data for the comparison of the contents of micro-nutrients (sterols and tocopherols) and inherent plant toxins (erucic acid) of refined oil of Liberator Phoe6/Ac plants and their controls have been obtained from pooled

samples of the one year's harvest from two different locations. Moreover, data submitted on the content of oil, protein, fatty acids, amino acids, erucic acid and glucosinolates have neither been analysed in a statistically sound way. In desolventising meal of Liberator Phoe6/Ac plants, which might be used as animal feed, the Dutch authority observed an increase of 20 to 25% of glucosinolates.

In 1999 the German authority determined refined oil of Liberator Phoe6/Ac substantially equivalent to that of its parental line Liberator regarding its nutritional value, intended use, composition and the level of undesirable substances. Protein content of the refined oil had been established at 0.01 mg/l based on information submitted in other, similar notifications of refined oil of Falcon GS40/90, Topas 19/2 and MS8xRF3. In June 2000, after questions raised by several member states, the applicant provided additional information on the three different methods that had been used for the analysis of the protein content of refined oil in these three different notifications. This information confirmed that no modified DNA or modified protein was present in refined oil of these three GM oilseed rape plants.

In November 2000 the EC Scientific Committee on Plants also concluded that GM oilseed rape varieties derived of transformation event Liberator Phoe6/Ac are substantially equivalent to their conventional counterparts except for the introduced traits.

4.2.2 *Falcon GS40/90 (AgrEvo/Aventis)*

The application file, which has been submitted under Directive 90/220 in Germany in 1996, contained a review of the compositional data by the Dutch research institute RIKILT dated on August 1999. In the view of RIKILT, the application had in first instance lacked a statistical analysis of the data on the oil, protein, fatty acid, and amino acid composition of seeds of GM oilseed rape plants. The application in first instance also lacked data on minerals or ash, vitamins (especially vitamin E) and data on the content of glucosinolates in seed and meal and on the content of sterols in the oil. The applicant subsequently provided additional information. Oil content of GM seeds was slightly but not consistently lower than in non-GM seeds, whereas in meal (for feed use) larger differences were found. The contents of individual glucosinolates in GM meal were lower in non-GM meal in most cases. RIKILT's final conclusion was that the GM seeds did not differ substantially from non-GM seeds except for the presence of a modified protein.

In July 1998 the EC Scientific Committee on Plants concluded that GM oilseed rape plants derived of Falcon GS40/90 are substantially equivalent to their unmodified counterparts except for the inserted traits. One year later, in July 1999, the German authority determined refined oil of Liberator Phoe6/Ac substantially equivalent to that of its 'comparator' regarding its nutritional value, intended use, composition and the level of undesirable substances. Protein content of the refined oil had been established at 0.01 mg/l like in other, similar notifications of refined oil of Topas 19/2 and MS8xRF3. In June 2000, after questions raised by several member states, the applicant provided additional information on the three different methods that had been used for the analysis of the protein contents of refined oil in these three different notifications. This information confirmed that no modified DNA or modified protein was present in refined oil of these three GM oilseed rape plants.

4.2.3 *MS8xRF3 (Plant Genetic Systems/AgrEvo/Aventis)*

The application file, which has been submitted under Directive 90/220 in Belgium in 1996, contained data on the content of oil, fibre, vitamins, minerals, glucosinolates and amino acids in desolventising meal of GM plants, control-plants and commercial varieties, and on the content of chlorophyll, tocopherols, sterols, minerals, fatty acids and erucic acid in processed oil. Samples were pooled from the harvest of one year at many locations. The data were not statistically analysed. In one case the applicant observed significant differences in levels of glucosinolates between RF3 plants and their controls. The applicant argued that these differences were due to environmental conditions. The Belgian authority concluded that MS8xRF3 is substantially equivalent.

Three years later, in November 1999, the German authority for Regulation 258/97 determined refined oil of MS8xRF3 substantially equivalent to that of its comparator regarding its nutritional value, intended use, composition and the level of undesirable substances. Protein content of the refined oil had been established at 0.01 mg/l like in other, similar notifications of refined oil of Topas 19/2 and Falcon GS40/90. In June 2000, after questions raised by several member states, the applicant provided additional information on the three different methods that had been used for the analysis of the protein contents of refined oil in these three different notifications. This information confirmed that no modified DNA or modified protein was present in refined oil of these three GM oilseed rape plants.

4.2.4 *Topas 19/2 (AgrEvo/Aventis)*

In June 1997, based on a compositional analysis of the content of oil, fatty acid, erucic acid and glucosinolates, the UK ACNFP concluded that the refined oil from Topas 19/2 did not differ in composition from conventional rape. However, as there was little experience in predicting the effect of genetic drift on the metabolism of any line of plants, whether GM or non-GM, the UK authorities asked the applicant to monitor the seed composition and the fatty acid profile of GM swede rape over time.

In February 1998 the EC Scientific Committee on Plants concluded that refined products from plants derived from Topas 19/2 would be safe for food use on the basis of its substantial equivalence. The data submitted concerned the content of oils, fatty acids, erucic acid and glucosinolates and were obtained from the harvest of trials at a number of locations in several successive years.

4.2.5 *Findings of the case studies on GM rape*

- Initial assessments by the German, respectively UK competent authority determined refined oil from Liberator Phoe6/Ac, Falcon GS40/90, MS8xRF3 and Topas 19/2 as substantially equivalent. The UK authority requested the applicant to monitor the seed composition and fatty acid profile of the oil of Topas 19/2 over time, as there was little experience in predicting the effect of genetic drift on the metabolism of any plant, whether GM or conventionally bred. In all these cases, the EC Scientific Committee on Plants also determined (refined oil of) these GM rape plants as substantially equivalent (except for the modified trait).

- However, in all cases differences in composition of the oil and/or the meal (for feed use) between the GM-plant and its non-GM control have been observed by member states. Further, from case to case, there has been a lack of consistency in compositional data submitted on the content of macro- and micronutrients, minerals, vitamins, inherent plant toxins and anti-nutrients. The content of sinapine, an anti-nutrient of rape, has not been determined in all cases. In addition, the design of the field trials, the number of locations and seasons, and the choice of the 'comparator' have considerably differed from case to case. It is hardly plausible that the compositional data have been analysed in a statistically sound way.

4.3 Analysis of the case studies on GM maize

4.3.1 *Bt11 silage maize (Novartis/Syngenta)*

In February 1998 the UK authority notified processed products of Bt11 *silage maize* (used for feed) to the European Commission. Its initial assessment report was based on data from the compositional analyses with regard to starch, protein, oil, fibre, fatty acids and amino acids. Although statistically significant differences in protein content were found between some GM hybrids and their controls, the conclusion was that Bt11 silage maize lines would not differ in composition from conventionally bred maize. However, as there was little experience in predicting the effect of genetic drift on the metabolism of any line of plants, whether GM or non-GM, the UK authorities asked the applicant to monitor the seed composition and the fatty acid and amino acid profile of the GM maize over time.

In February 1998, based on data on the content of oil, fatty acids, fibre, starch and amino acids obtained through compositional analysis of seeds from trials at a number of locations, the EC Scientific Committee on Plants concluded that products of imported Bt11 maize are substantially equivalent.

In August 2000 the Italian authority informed the European Commission that it considered Bt11, MON810 and T25 not to be substantially equivalent within the meaning of Regulation 258/97 and should therefore be submitted for a full safety evaluation. According to Italy, the original application lacked PCR data to detect modified DNA in products of these GM maize plants. In September 2000 the EC Scientific Committee on Food however viewed that there were no scientific grounds for considering that the use of these novel foods would endanger human health.

Three months later, in November 2000, the EC Scientific Committee on Plants delivered its opinion on Bt11 maize. It noted several (minor) differences. In its opinion the SCP did not explicitly determine Bt11 maize as substantially equivalent.

4.3.2 *Bt11 sweet maize (Novartis/Syngenta)*

In April 1998 the Dutch authority received a request for an *authorisation* of fresh and processed kernels of Bt11 *sweet maize* (used for food). The applicant provided data on the content of starch, protein, oil, fibre, fatty acids and amino acids. The Dutch

authority however wished additional data on the contents of the five secondary metabolites to underpin the degree of substantial equivalence: furfural, p-coumaric acid, ferulin acid, raffinose and phytic acid. These secondary metabolites were selected because their concentration range in maize was known and because furfural's potential carcinogenicity. The company provided these data in January 2000. In April 2000 the Dutch authority issued its initial assessment report, which led to several questions from other member states. In November 2000 the company provided a response to these questions.

The initial assessment by the Dutch authority treated the data submitted in a rather critical way. It for example observed significant differences with regard to some nutrients. It also expressed the view that the study on the contents of minerals and vitamins should not have pooled the data obtained from several locations. The analysis of contents of the five secondary metabolites was based on samples taken from three Bt11 hybrids and their controls grown at one location. No statistically significant differences have been found. In its initial assessment report the Dutch authority did not unambiguously establish the (degree of) substantial equivalence of Bt11.

Several member states argued for several reasons that the data submitted were insufficient to establish the substantial equivalence of Bt11. In addition, several member states viewed the statistical analysis of the data inadequate. In its response the company acknowledged that the use of various hybrids, different experimental designs and different analytical methods makes a direct comparison of the numerous compositional analyses difficult. In the view of the applicant, this was due to the fact that the requirements for compositional analyses evolved between 1995 and 2000. In its response the company also provided an extensive justification for the choice of compounds, which have been analysed.

At the moment, it is not clear whether the applicant's response satisfies the other member states, nor is it clear how this impacts regulatory decision-making on the food use of Bt11 sweet maize by the European Commission and the national authorities of EU member states.

4.3.3 GA21 (*Monsanto*)

In July 1998 the applicant requested the Dutch authority to *authorise* GA21 for food use and provided data on Ash, calcium, carbohydrates, acid detergent fibre ADF, neutral detergent fibre NDF, moisture, phosphorous, protein, fat, fatty acids, amino acids, trypsin inhibitors, phytic acid, and vitamin E.

In February 1999 the applicant was requested to provide additional information about the contents of five secondary metabolites furfural, p-coumaric acid, ferulin acid, raffinose and phytic acid. In March 1999 the company argued that it would not be relevant to investigate the levels of these secondary plant metabolites, as maize has a safe history of use. In June 1999 the Dutch authority reiterated its request for additional information, which was provided by the company in October 1999. In December 1999 the Dutch issues its initial assessment report.

The initial assessment by the Dutch authority treated the data submitted in a rather critical way. It noted for example that the level of p-coumarin acid was significantly

higher in one of the GA21 hybrids. The applicant argued that this difference was small, fell within the range of values for other commercial maize varieties and was unlikely to be biologically significant. The Dutch authority also noted that applicants would be helped by concrete guidance concerning the number of samples, locations and years, which would be needed for the quantitative analyses. Nonetheless, on the basis of these data (and on data with respect to the newly introduced DNA and protein) submitted, the Dutch authority concluded that consumption of GA21 maize is as safe for humans as non-GM maize. But the Dutch authority did not unambiguously establish the (degree of) substantial equivalence of GA21.

Several member states argued for several reasons that the data submitted were however insufficient to establish the substantial equivalence of GA21. In addition, several member states viewed the statistical analysis of the data inadequate. At the moment, it is not clear whether the questions raised by other member states have been responded, nor is it clear how this impacts regulatory decision-making on the food use of GA21 maize by the European Commission and the national authorities of EU member states.

In September 2000 the EC Scientific Committee on Plants determined that GA21 maize is substantially equivalent to its conventional counterparts (except for the modified trait).

4.3.4 T25 (*AgrEvo/Aventis*)

In January 1998, on the basis of data on the contents of fatty acids, protein, amino acids, crude fibre, ash, phytate and moisture, the UK authority concluded that there had been no unintentional changes in the composition of T25 maize. But for some parameters variation between the GM and non-GM lines had been observed. Further, as there was little experience in predicting the effect of genetic drift on the metabolism of any line of plants, whether GM or non-GM, the UK authority asked the applicant that the seed composition and the fatty acid and amino acids profiles of T25 maize lines should be monitored over time.

According to the EC Scientific Committee on Plants in February 1998, integration and expression of the gene did not seem to cause any negative pleiotropic effects. The nutritional composition and the content of anti-nutrients in T25 were within the normal range. It concluded that T25 is substantially equivalent to its non-GM counterpart except for the introduced trait.

In August 2000 the Italian authority informed the European Commission that it considered Bt11, MON810 and T25 not substantially equivalent within the meaning of Regulation 258/97 and should therefore be submitted for a full safety evaluation. According to Italy, the original application lacked PCR data to detect modified DNA in products of these GM maize plants. In September 2000 the EC Scientific Committee on Food however viewed that there were no scientific grounds for considering that the use of these novel foods would endanger human health.

4.3.5 *MON810 (Monsanto)*

In December 1997 the UK authority notified processed products of MON810 to the European Commission. The applicant provided data on the content of fatty acid, protein, amino acid, crude fibre, ash and moisture content of grain and forage GM and non-GM maize grown in agronomic fields tests in Europe and in the USA. According to ACNFP, there had been no unintentional changes in the composition of the grain from the GM plants or in the plants themselves as a result of the genetic modification. It concluded that the composition of the GM maize does not differ from that of conventionally bred maize. However, there was little experience in predicting the effect of genetic drift on the metabolism of any line of plants, whether genetically modified or conventionally bred. Therefore ACNFP asked the applicant to monitor the seed composition, including amino acid and fatty acid profiles of the oil from MON810 over time.

In February 1998, based on compositional analysis with regard to fatty acids, protein, amino acids, crude fibre, ash, phytic acid and moisture, the EC Scientific Committee on Plants determined MON810 to be substantial equivalent to non-GM maize except for the introduced traits.

In August 2000 the Italian authority informed the European Commission that it considered Bt11, MON810 and T25 not substantially equivalent within the meaning of Regulation 258/97 and should therefore be submitted for a full safety evaluation. According to Italy, the original application lacked PCR data to detect modified DNA in products of these GM maize plants. In September 2000 the EC Scientific Committee on Food however viewed that there were no scientific grounds for considering that the use of these novel foods would endanger human health.

4.3.6 *Conventional crosses of T25 and MON810 (Pioneer Hi-Bred)*

In 1999 the UK authority received an application for a maize hybrid derived from conventional crosses of two GM maize lines approved under Directive 90/220, probably T25xMON810. According to the UK authority, the applicant had heavily relied on the fact that these two GM parents had already been approved, so that very little information on the new hybrid was provided. The assumption that this hybrid would exhibit the same properties as the two GM parent lines was challenged. In particular, the possibility of unintended effects arising from insertion events brought about by the crossing could not be ruled out. At this stage such crosses would need to be assessed on a case by case basis. The UK authority also noted that in other countries, such as the USA, clearance of a GM line also covered further crosses with approved GM lines. It seems however that the application has been withdrawn from the UK.

In April 2000 a request for authorisation of processed products of conventional crosses between T25 and MON 810 maize hybrids was submitted in The Netherlands. The Dutch authority has not yet completed its initial assessment report.

In June 2000, on the basis of compositional analysis of the content of protein, fibre, fat, moisture, ash, fatty acids, amino acids, Ca, K, Mg, Mn, Na, P, folic acid, thiamine, riboflavin, vitamin E, phytic acid and trypsin inhibitors, the EC Scientific Committee

on Plants noted significant differences for grain ash, stearic acid, linolenic acid, arginine and riboflavin between T25xMON810 and its control but their levels were within ranges published for maize. It concluded that T25xMON810 was substantially equivalent to its parental lines T25 and MON810.

4.3.7 Findings of the case studies on GM maize

- The initial assessments for notification of Bt11 silage maize, T25 and MON810 by the UK authority determined that these GM maize plants did not differ in composition from their non-GM controls. As there was little experience in predicting the effect of genetic drift on the metabolism of any line of plants, whether GM or non-GM, the UK authorities asked the applicant to monitor the seed composition and the fatty acid and amino acid profile of the GM maize over time. In 1998 the EC Scientific Committee on Plants determined that Bt11 (silage) maize as substantially equivalent, whereas in 2000 this committee did not explicitly reach the conclusion that it should be viewed as substantially equivalent. T25 and MON810 were both determined to be substantially equivalent (except for the modified trait) by the EC Scientific Committee on Plants.
- However, in all these cases of notifications differences in the composition of the GM maize plant and its non-GM control have been observed. Further, from case to case, there has been a lack of consistency in compositional data submitted on the content of macro- and micro-nutrients, minerals, vitamins, inherent plant toxins and anti-nutrients. For example, the content of trypsin inhibitor, an anti-nutrient in maize, has not been determined in all cases. In addition, the design of the field trials, the number of locations and seasons, and the choice of the 'comparator' have considerably differed from case to case. It is hardly plausible that the compositional data have been analysed in a statistically sound way.
- The requests for authorisation of Bt11 *sweet* maize and GA21 have been submitted to the Dutch authorities, which treated the data submitted in a rather critical way. Applicants were urged to provide additional data on the content of five secondary metabolites in the GM maize plants compared to their non-GM controls to underpin the degree of substantial equivalence. Such data were provided, but it did not lead unambiguously to a determination of (the degree of) substantial equivalence of GA21. The Dutch authorities noted that applicants would be helped by concrete guidance concerning the number of samples, locations and years, which would be needed for the quantitative analyses.
- In both these cases of a 'heavy' authorisation procedure, several member states raised critical questions on the assessment by the Dutch authorities. In the case of Bt11 *sweet* maize the applicant has provided a response to these questions. In the case of GA21 it is not clear whether the applicant has provided additional information. In both cases it is unclear how these questions and responses impact regulatory decision-making by the European Commission and national authorities of EU member states, as the EC Scientific Committee on Plants has determined Bt11 (*sweet*) maize as well as GA21 maize as substantially equivalent to their non-GM controls.

- The request for authorisation of crosses of T25 and MON810 has been submitted to the Dutch authorities, which have not yet completed their initial assessment. The EC Scientific Committee on Plants concluded that T25xMON810 hybrids are substantially equivalent to T25 and MON810 and non-GM maize.

4.4 Analysis of the case study on GM tomato TGT7F

4.4.1 TGT7F (*Zeneca/Syngenta*)

The UK authority received the original submission in August 1994. In February 1995 the UK authority approved the food use of paste of two GM tomato hybrid lines. In August 1995 the applicant requested to extend the scope of the original approval to tomato paste and processed tomato products containing the paste, like ketchup and pizza sauces. The request was also forwarded to extend the approval to any tomato line containing the construct pJR16S. The UK authority agreed with extending food safety clearance to paste and products containing the paste but not to any line containing the construct.

Comparative nutritional and toxicological analyses were carried out on GM tomatoes and commercially available, conventional tomato varieties produced during trials in one year. Nutritional values fell within the range of controls and the data on four known inherent tomato toxins (alpha-tomatine, solanine, chaconine and nicotine) indicated that their levels in both the GM and non-GM samples were below the level of detection. The UK authority concluded that the nutritional and toxicological composition of the GM tomato was comparable to that of conventional tomatoes.

In 1997 Regulation 258/97 came into force. The applicant submitted a request for a safety evaluation of TGT7F by the UK authority in March 1998. Data were provided on the content of moisture, ash, fat, total carbohydrate, total dietary fibre, soluble fibre, insoluble fibre, total sugars, protein, cellulose, hemicellulose, energy value, Na, K, Ca, Mg, P, Fe, vitamins A, E, B1, B2, B6, C, niacin, folic acid, malic acid, citric acid and lycopene. On this basis the UK authority concluded that the genetic modification had not changed the nutritional profile. Further, other inherent tomato toxins, such as tyramine, serotonin, histamine, nicotine and lectins, were also analysed. Their levels in GM tomato paste were comparable to those in the controls. In an exemplary case study on the application of the concept of substantial equivalence by the OECD in 1993, it was suggested to include inherent tomato toxins, like alpha-tomatine, tomatidine, aglycone of tomatine, saponines, coumarins, lectins, serotine, oxalate, protease-inhibitor and histamine

Levels of toxic metals (As, Cd, Pb and Hg) in the GM tomato fell within the range of values obtained for its control. It was further concluded that the NPTII gene encoding for kanamycin-resistance and its protein, which could not be detected in processed product from the GM tomato, were not inherently toxic. According to the UK authority, no nutritional and toxicological differences existed between the GM tomato and its non-GM counterpart. This was seen as further evidence that no secondary effects had occurred as a result of the genetic modification.

In June 1998 the EC Scientific Committee on Plants concluded that the harvested GM tomato fruit is substantially equivalent to fruit of other commercial processing tomatoes.

In September 1999 the EC Scientific Committee on Food confirmed the opinion of the SCP that the GM tomato is substantially equivalent to unmodified and conventional counterparts except for the introduced traits.

4.4.2 Findings of the case study on GM tomato TGT7F

- The applicant sought a (full) safety evaluation of processed products of GM tomato TGT7F. The UK authority concluded that no nutritional and toxicological differences existed between the GM tomato and its control. The UK authority did not explicitly establish the GM tomato as substantially equivalent, whereas the EC Scientific Committee on Plants and the EC Scientific Committee on Food both concluded that the GM tomato is substantially equivalent.
- Compositional data have been obtained from trials during one year. It is not clear whether these data have been analysed in a statistically sound way.
- Comparative data of the GM tomato and its control regarding several inherent tomato toxins, such as tomatidine, aglycone of tomatine, saponines, coumarins, protease-inhibitor and oxalate, were not provided.

ANNEX I

HISTORY OF THE CONCEPT OF SUBSTANTIAL EQUIVALENCE

OECD, 1993

In the early 1970s the first so-called “recombinant DNA” (rDNA) techniques were developed for splicing DNA molecules and reintroducing them into living organisms. Some molecular biologists immediately expressed concern about specific dangers from the new tools, which might allow production of harmful viruses on a much larger scale than would otherwise be possible, or to transfer genes for antibiotic resistance into disease-causing bacteria, thereby rendering antibiotic therapy ineffective. This prompted the US National Academy of Science to convene a committee that was chaired by Paul Berg. This committee distributed a letter, (sometimes referred to as the “Berg” letter) calling for a voluntary moratorium on the cloning of genes for antibiotic resistance genes and of DNA from animal viruses pending an international conference to discuss safety issues related to these rDNA techniques. That conference¹ was held at Asilomar, California, in 1975, which set in motion the first discussions on how to regulate the safety of recombinant DNA research in the US and later in almost every industrialised country.

A couple of years later rDNA techniques were used to develop novel organisms for experiments and usage outside laboratories. In 1983 this led the Committee for Scientific and Technology Policy of the Organisation of Economic Co-operation and Development (OECD) to create a Group of National Experts on Safety in Biotechnology (GNE). In 1986 the work of the GNE resulted in general safety guidelines² for the use of genetically modified organisms (GMOs) in industry, agriculture and the environment. These guidelines recommended focusing safety concerns on whether organisms modified by recombinant DNA techniques pose an ‘incremental’ risk to organisms modified by conventional means. While rDNA techniques may result in the production of organisms expressing a combination of traits that are not observed in nature, genetic changes from rDNA techniques will often have inherently greater predictability compared to traditional techniques, because of the greater precision that rDNA technique affords to particular modification. On this conceptual basis³ the GNE expected that any risk associated with the application of rDNA modified organisms may be assessed in generally the same way as those associated with non-rDNA organisms.

Four years later, in 1990, the GNE agreed to establish a Working Group for developing scientific principles that would focus on the safe use of new food or food components derived by modern biotechnology. In 1993 OECD⁴ published the results, which indicated that the “scientific approach to safety evaluations of new foods or food components derived by modern biotechnology (...) is based on a comparison with traditional foods that have a safe history of use. This approach is based in turn on the concept of substantial equivalence, which articulated procedures used in the past, albeit intuitively, for accepting new foods”. According to the report, the safety of food for human consumption is based on the concept that there should be a reasonable certainty that no harm will result from intended uses under

¹ See for instance: *The Genetic Revolution: scientific prospects and public perceptions*, ed. Bernard D. Davids, The John Hopkins University Press, 1991.

² OECD, *Recombinant DNA Safety Considerations*, 1986.

³ There are however scientific controversies surrounding this conceptual basis: 1) De Visser, A.J.C., et al., *Crops of Uncertain Nature?, Controversies and Knowledge Gaps Concerning Genetically Modified Crops*, Report 12, Plant Research International Wageningen University Research, Commissioned by Greenpeace Netherlands, Wageningen, August 2000, and; 2) Regal, P.J., *Scientific principles for ecologically based risk assessment of transgenic organisms*, *Molecular Ecology*, Vol. 3, pg. 5-13, 1994.

⁴ OECD, *Safety evaluation of foods derived by modern biotechnology: concepts and principles*, 1993. The report focused on genetically modified organism of microbial, plant and animal origin; organisms of aquatic origin were to be addressed in future work.

the anticipated conditions of consumption. Historically, foods prepared and used in traditional ways have been considered safe on the basis of long experience even though they may have contained natural toxicants or anti-nutritional components. According to the working group, modern biotechnology broadens the scope of the genetic changes that can be made in food organisms. This does not inherently lead to foods that are less safe than their conventional counterparts and therefore safety evaluations of foods derived by modern biotechnology do not necessitate a fundamental change in established principles, nor do they require a different standard of safety. Moreover, the precision inherent in the use of certain molecular techniques should enable direct and focussed safety assessment where such assessment is desired. In the view of the Working Group, the most practical approach to the determination of safety of foods derived by modern biotechnology is to consider whether they are substantially equivalent to analogous conventional food product(s), if such exists. A demonstration of substantial equivalence takes into consideration a number of factors, such as:

- Knowledge of the composition and characteristics of the traditional or parental product or organism.
- Knowledge of the characteristics of the new component(s) or trait(s) derived, as appropriate, from information concerning: the component(s) or trait(s) as expressed in the precursor(s) or parental organisms; transformation techniques (as related to understanding the characteristics of the product) including the vector(s) used and any marker genes used; possible secondary effects of the modification; and the characterisation of the component(s) or trait(s) as expressed in the new organism.
- Knowledge of the new product/organism with the new component(s) or trait(s), including the characteristics and composition [i.e. the amount of the components or the range(s) of the expression(s) of the new trait(s)] as compared with the conventional counterpart(s) (i.e. the existing food or food component).

In addition, the principle was set out that if a modified food or food component is determined to be substantially equivalent to an existing food, it can be treated in the same manner. No additional safety concerns would be expected. However, when a product is determined not to be substantially equivalent, the identified differences should be focus of further evaluations. In case there is no basis for comparisons, that is, where no counterpart has been previously consumed as food, the new food or food component should be evaluated on the basis of its own composition and properties.⁵

FAO and WHO, 1996

In 1996, three years after the OECD had introduced the concept of substantial equivalence an expert consultation was convened jointly by the Food and Agriculture Organisation (FAO) and World Health Organisation (WHO). This Consultation⁶ recommended – in line with OECD - that substantial equivalence is an important component in the safety assessment of foods and food ingredients derived from genetically modified plants intended for human consumption.

Beyond 'substantial equivalence', Nature, October 1999

On October 7th, 1999, the authoritative scientific journal Nature⁷ published a critical view on the concept of substantial equivalence. In the authors' view, the degree of difference between a natural food and its GM alternative before its 'substance' ceases to be acceptably 'equivalent' had not been not defined, nor had an exact definition been agreed by legislators. The vagueness of this concept might make the concept useful to industry but unacceptable to the consumer. The authors therefore argued that legislators should have treated GM foods in

⁵ A number of case studies illustrated the practical application of the concept of substantially equivalence and the principles developed by the Working Group. The case study on genetically modified tomato will be discussed in this report's case study on 'Transformation event TGT7F'.

⁶ Report of a joint FAO/WHO consultation, FAO Food and Nutrition Paper 61, Food and Agriculture Organisation of the United Nations, Rome, 1996.

⁷ Millstone, E., Brunner, E. and Mayer, S., Beyond "substantial equivalence", Nature, October 7, 1999.

the same way as novel chemical compounds such as pharmaceuticals, pesticides and food additives. Companies should have been required to conduct a range of toxicological tests to set 'acceptable daily intakes' (ADIs). Although the authors acknowledged that toxicological and biochemical tests, and their interpretation, are notoriously problematic and contested, and are slow and expensive, they could provide information vital to consumer protection.

Subsequently, criticism was expressed that the concept of substantial equivalence had been misapplied in the case of genetically modified, glyphosate-tolerant soybeans⁸, even on its own terms. With reference to a report⁹ by a Dutch government team, the authors reached the final conclusion that "substantial equivalence is inherently anti-scientific because it was created primarily to provide an excuse for not requiring biochemical and toxicological tests." They ended by recommending "to replace the concept with a practical approach which would actively investigate the safety and toxicity of GM foods rather than merely taking them for granted".

The OECD responded^{10,11} that the substantial equivalence was not developed as an excuse for not requiring biochemical or toxicological tests but emerged from a number of observations. The first and most significant of these was that new varieties of whole foods coming to the market do not undergo extensive toxicological testing. The second observation was the knowledge that traditional whole foods often contain natural toxicants and/or anti-nutritional substances, so that it would be likely that a battery of traditional toxicological tests might well reveal undesirable effects in animal tests. Despite this, whole foods if prepared and used in appropriate ways have been considered safe on the basis of long term experience.

The OECD further argued that in assessing the safety of a GM food, it would be unreasonable and unscientific to expect an absolute standard of safety, which could be demonstrated through traditional testing. Therefore a notion was required that a new modified variety should be as safe as traditional varieties. So the important starting point in the safety assessment should be a comparison between the new genetically modified variety and its traditional counterpart, that is a determination of substantial equivalence. OECD emphasised that substantial equivalence is not in itself a substitute for a safety assessment. It is a guiding principle, which is intended to be a useful tool for those engaged in safety assessments. Finally, the OECD pointed out that there is work under way to identify the critical nutrients and

⁸ Glyphosate-tolerant soybeans not sprayed with glyphosate have been deemed to be substantially equivalent to non-GM soybeans, according to the authors, by (...) focusing on a restricted set of variables, such as the amounts of protein, carbohydrate, vitamins and minerals, amino acids, fatty acids, fibre, ash, isoflavones and lecithins, although application of glyphosate changes their chemical composition, e.g. the level of phenolic compounds such as isoflavones. A response of a Group of Monsanto scientists, published by AGNET, Canada, October 5th, 1999, suggested that data from comparative compositional analyses of GT-soybean not treated with glyphosate and non-GM soybeans had been published in 1996, while data on GT-soybean treated with glyphosate had been published in 1999. These studies demonstrated no significant differences regarding key soybean nutrients and anti-nutrients, including phytoestrogen levels. According to a Dutch study by RIKILT commissioned by the Foundation "Consument en Biotechnologie" (March 2000), the CP4 EPSPS enzyme in GT-soybean may alter the amino acid biosynthesis but no significant differences regarding amino acid composition have been found in GT-crops on the market. The study also suggested that levels of phenolic compounds may also be affected in plants genetically modified with this enzyme.

⁹ Kuiper, H.A., et al., Food Safety Evaluation of Genetically Modified Foods as a Basis for Market Introduction, Ministry of Economic Affairs, 1998.

¹⁰ OECD, The concept of substantial equivalence in the safety assessment of novel foods, Food Industry Environmental Network (FIEN), October 7th, 1999.

¹¹ Several authors have expressed their disagreement with Miller *et al.* (1999) in a number of letters and commentaries. See for instance: 1) Gasson, M.J., Genetically Modified Foods Face Rigorous Safety Evaluation, *Nature*, Vol. 402, pg. 229, 1999; 2) Miller, H.I., Substantial equivalence: Its Uses and Abuses, *Nature Biotechnology*, Vol. 17, pg. 1042-1043, 1999; 3) Tester, M., *et al.*, Seeking Clarity in the Debate over the Safety of GM Foods, *Nature*, Vol. 402, pg. 575, 1999; and 4) Trewavas, A. and Leavert, C.J., Conventional Crops are the Test of GM Prejudice: Kearns, P., and P. Mayers, Substantial Equivalence is a Useful Tool; Burke, D. No GM Conspiracy, *Nature*, Vol. 401, pg. 640-641, 1999.

toxicants found in major crop plants, which would be an important focus of a demonstration of substantial equivalence.

OECD Edinburgh Conference, February/March 2000

In his report¹² the chairman of the OECD Edinburgh Conference on 28 February and 1 March 2000 concluded that safety assessment of any novel food involves a variety of kinds of evidence. One commonly used tool was the concept of substantial equivalence. The essence of this idea was that a comparison between the novel food and one already in the diet provides the basis for asking questions about the safety of the novel products. Substantial equivalence was not a quantitative criterion or hurdle. According to the report, it was continually and updated, but it was timely now, after six years of using the tool, to undertake a more detailed review.

FAO and WHO Codex Alimentarius, March 2000

In June/July 1999 the Codex ad hoc Intergovernmental Task Force on Foods Derived from Biotechnology had been established by the twenty-third session of the FAO/WHO Codex Alimentarius Commission. Nearly a year later, in March 2000, in its first session, this Task Force¹³ welcomed the initiative of FAO and WHO to convene another joint expert consultation two months later, in May/June. The Task Force also identified, among others, the following questions for this joint expert consultation: What is the role, and what are the limitations, of substantial equivalence in the safety and nutritional assessment? And are there alternative strategies to substantial equivalence that should be used for the safety and nutritional assessment?

OECD, May 2000

In May 2000 the OECD Task Force for the Safety of Novel Foods and Feeds reported¹⁴ that in 1996 an FAO/WHO consultation had endorsed the application of substantial equivalence. It had recognised that establishment of substantial equivalence is not a safety assessment *per se*, nor intended to be a measure of absolute safety. Establishment of the new GM food as substantially equivalent implies that it will be no less safe than the conventional food, while demonstrating absolute safety is an impractical goal. A key criticism had been that substantial equivalence is not measurable and therefore inappropriate to safety assessment. However, in the view of the Task Force, the concept of substantial equivalence is a tool, which helps to identify any difference, intended or unintended, that might be focus of further safety evaluation, involving traditional nutritional, toxicological or immunological testing, or long-term studies, depending on the identified differences. Determining substantial equivalence entails consideration of:

- The trait encoded by the genetic modification.
- Phenotypic characterisation of the new food source, compared with an appropriate comparator already in the food supply.
- Compositional analysis of the new food source or the specific food products compared with the selected comparator.

Because it is a comparative process for safety evaluation, the determination of substantial equivalence can be performed at several points along the food chain (e.g. at the level of the harvested or unprocessed food product, individual processed fractions or the final food product or ingredient). Although from a practical point (e.g. where multiple fractions from a

¹² GM Food Safety: Facts, Uncertainties, and Assessment, The OECD Edinburgh Conference on the Scientific and Health Aspects of Genetically Modified Foods, 28 February – 1 March 2000.

¹³ Report of the first session of the Codex ad hoc Intergovernmental Task Force on Foods derived from Biotechnology, Chiba, 14 – 17 March, 2000.

¹⁴ Report of the Task Force for the Safety of Novel Foods and Feeds, OECD, C(2000)86/ADD1, May 17th, 2000.

single source will be used as different food products) substantial equivalence should be typically determined at the level of the unprocessed food product, the flexibility of the concept permits the determination to be targeted at the most appropriate level, based upon the nature of the product.

The Task Force further discussed why the choice of an appropriate comparator having an acceptable history of safe food use is crucial to effective application of the concept of substantial equivalence of a new GM food. Sufficient analytical data should be available in literature, or be generated through analysis, to allow a comparison to be made. Guidance on applying the concept had been developed to provide clarity in interpreting the criteria that would constitute an appropriate framework for determining substantial equivalence. The Task Force also pointed out that the nature of the comparative approach with respect to nutrients limits its universality, since the relevance of nutrients in a particular crop depends on consumption patterns that may vary from region to region. Therefore, where such differences exist, these must also be considered in identifying the key nutrients to be assessed.

FAO and WHO, May/June 2000

The FAO/WHO joint expert consultation¹⁵ in 2000 noted that since 1996 several countries had used the concept of substantial equivalence and had found this approach to be scientifically sound and practical. However there had not been universal consensus on the application of this concept. This had resulted in criticism that substantial equivalence did not provide a sufficient basis for safety and in calls from national governments and international bodies to consider alternative approaches. In 2000 FAO and WHO therefore convened another expert consultation to review the scientific basis, application, and limitations of the concept of substantial equivalence.

One of the papers¹⁶ discussed by the Consultation summarised differences between chemical and food toxicity evaluation in the following table.

CHEMICAL	FOOD
Material usually simple, chemically precise substance	Complex mixture of many compounds
Highest dose level should produce an effect	Effects improbable at the maximum dose level that can be incorporated in the diet for the test species
Small dose (usually less than 1% of diet)	High intake (usually greater than 10%)
Acute effects obvious	Acute effects difficult to produce (usually absent)
General independent of nutrition	Nutrition dependent
Specific route of metabolism simple to follow	Complex metabolism
Cause/effect clear	Cause/effect, if observed at all, may be confused

The Consultation argued that in contrast to many compounds such as pesticides, pharmaceuticals, industrial chemicals and food additives, whole foods are complex mixtures of compounds characterised by wide variation in composition and nutritional value. Practical difficulties of obtaining meaningful information from conventional toxicology studies on the safety of whole foods, which had become particularly apparent when animal feeding studies were used to assess the safety of irradiated foods, meant that an alternative approach was required for the safety assessment of genetically modified foods. This has led to the development of the concept of substantial equivalence. The Consultation agreed that the practical difficulties in the application of conventional toxicology studies to whole foods preclude their use as a routine safety assessment technique for genetically modified foods.

¹⁵ Safety aspects of genetically modified foods of plant origin, Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, 29 May – 2 June 2000.

¹⁶ Topic 1: The concept of substantial equivalence, its historical developments and current use, Nick Tomlinson, Foods Standards Agency, United Kingdom, Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, 29 May – 2 June 2000.

Further, from an animal welfare perspective the Consultation recognised that the use of toxicology studies could not be justified where it was unlikely to result in meaningful information. It also noted that the concept of substantial equivalence was a good example of an approach to reduce the use of animals in toxicology studies by refining safety assessment techniques and replacing animal models with alternatives.

The paper¹⁷ 'Beyond substantial equivalence' published by Nature in October 1999 was also presented at the meeting. The Consultation acknowledged that the concept of substantial equivalence had attracted criticism. In part, this related to the mistaken perception that determination of substantial equivalence was the end point of safety assessment rather than a starting point. It is a concept used to identify similarities and differences between the genetically modified food and its traditional counterpart. Intended and unintended differences identified then become focus of the safety assessment. The potential occurrence of unintended effects is not specific to the use of recombinant DNA techniques, in the view of the Consultation. It is an inherent and general phenomenon that can occur in conventional breeding, in which one of the approaches to cope with this problem is to select and discard plants with unusual and undesired phenotypic and agronomic parameters at an early stage of plant variety development. Consecutive back-crossing is also a common procedure to eliminate unintended effects. Unintended effects due to genetic modification may be 'predictable' based on metabolic connections to the intended effect or knowledge of the site of insertion, or they may be 'unexpected'.

The Consultation further indicated that the comparator used to detect unintended effects should ideally be the near isogenic parental lines grown under identical conditions. In practice, this may not be feasible at all times, in which case a line as close as possible should be assessed. The resulting natural variation should be taken into account in assessing the statistical significance of the unintended effect. Where statistically significant unintended effects are observed, their biological significance and safety should be assessed. For that purpose, data on the genetically modified plant should be compared to data on other conventional varieties and literature data. If the differences exceed natural variations in traditional food crops, further assessment is required. Data for comparison should be obtained using validated methods and analysed using appropriate statistical techniques.

One of the papers¹⁸ discussed by the Consultation indicated that applicants for marketing of food produced from GMOs had normally used the comparison principle of substantial equivalence but there had been a lack of consistency from case to case in the data provided, even within the same plant species. This inconsistency and sometimes lack of data demonstrated the need for guidelines in order to harmonise the basis data requirements for comparison. The paper recommended therefore developing consensus documents and/or databases for different species or organisms and foods and feeds. Guidelines should also be developed for key substances, nutrients and data needed for assessment of substantial equivalence including for each substance the variation related to the history of the food and feed and its intake. In addition, guidelines should be developed for appropriate design of field trial with GM food crops and for appropriate statistical methods.

The Consultation further indicated that present approaches to assess possible unintended effects are based, in part, on the analysis of specific components (targeted approach). In order to increase the probability of detecting unintended effects, profiling techniques are used at different level, e.g. genomics, proteomics and metabolomics, and may contribute to the

¹⁷ Topic 3: Limitation of substantial equivalence regarding the assessment of foods derived from modern biotechnology, Eric Brunner, University College London, United Kingdom, Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, 29 May – 2 June 2000.

¹⁸ Topic 2: Application of substantial equivalence, data collection and analysis, Jan Pedersen, Danish Veterinary and Food Administration, Denmark, Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, 29 May – 2 June 2000.

detection of differences in a more extensive way than targeted chemical analysis. However, they are not yet fully developed and validated and have certain limitations.

Finally, the Consultation was also of the view that there were no alternative strategies that would provide a better assurance of safety of genetically modified foods than the appropriate use of the concept of substantial equivalence. Some steps in the food safety assessment could nevertheless be refined by further developing and validating the new profiling techniques, as these would provide the means of a more detailed comparison.

Science and regulation of GM plants, United States National Research Council, 1999

In the fall of 1999 the US National Research Council published a first Internet version of a report¹⁹ by an expert committee that had discussed the science and regulation of genetically modified plants. The expert committee first task was to review the three principles formulated by the National Academy of Science (NAS) in 1987:

1. There is no evidence that unique hazards exist either in the use of rDNA techniques or in the movement of genes between unrelated organisms.
2. The risks associated with the introduction of rDNA-engineered organisms are the same in kind as those associated with the introduction of unmodified organisms and organisms modified by other methods.
3. Assessment of the risks of introducing rDNA-engineered organisms into the environment should be based on the nature of the organism and the environment into which it is introduced, not on the method by which it was produced.

The committee pointed out that it was not aware of controlled field studies, which directly compare the ecological effects of transgenic and conventional pest-protected plants bred for the same pesticidal trait. The committee indicated that its conclusions about these principles were therefore not based on data from such comparisons, but on mechanistic knowledge and scientific information about the resulting GM plants. It further argued that both conventional breeding and genetic modification have the potential to produce organisms of high or low risk, and therefore the properties of the organism should be the focus of risk assessment, and not the process by which it is produced.²⁰ With respect to the potential that health impacts of the consumption of GM plants may occur due to pleiotropic effects of genetic modifications, the committee concluded that monitoring of pleiotropic changes in plant physiology and biochemistry should be an important element of health safety reviews. Therefore it recommended research to assess and enhance data on the baseline concentrations of plant compounds of potential dietary or other toxicological concern, and to determine how concentrations of these compounds may vary depending on the genetic background of the plant and environmental conditions. It also recommended that the three regulatory agencies Environmental Protection Agency (EPA), Food and Drug Administration (FDA)^{21, 22} and United

¹⁹ Genetically Modified Pest-Protected Plants: Science and Regulations, Committee on Genetically Modified Pest-Protected Plants, Board on Agriculture and Natural Resources, National Research Council, National Academy Press, Washington, D.C., 2000 (See also <http://www.nap.edu>)

²⁰ To the opinion of Schenkelaars Biotechnology Consultancy, it could therefore be argued that conventionally bred plants should be subject to the same level of regulatory oversight as GM plants.

²¹ According to the Alliance for Bio-Integrity (1999), FDA's records reveal it declared genetically engineered foods to be safe in the face of broad disagreement from its own experts – all the while claiming a broad consensus supported its stance. Internal reports and memoranda disclose: 1) agency scientists repeatedly cautioned that foods produced through recombinant DNA technology entail *different* risks than do their conventionally produced counterparts, and 2) that this input was consistently disregarded by the bureaucrats who crafted the agency's policy, which treats bioengineered foods the same as natural ones. For instance, Dr Linda Kahl, an FDA compliance officer, objected that the agency was "trying to fit a square peg into a round hole ... [by] trying to force the ultimate conclusion that there is no difference between foods modified by genetic engineering and foods modified by traditional breeding practices. (See <http://www.bio-integrity.org>).

²² FDA's completed consultations on bioengineered foods indicate that MS8xRF3 rape plants, Bt11 maize plants, GA21 maize plants and T25 maize plants do not materially differ in composition, safety or

States Department of Agriculture (USDA) collaborate on the establishment of a database for natural plant compounds of potential dietary or other toxicological concern.

Elements of Precaution, the Royal Society of Canada, 2001

In February 2001, under the heading 'Elements of Precaution', an expert panel convened by the Royal Society of Canada²³ published recommendations for the regulation of food biotechnology in Canada. In relation to the concept of substantial equivalence, the expert panel noted that conceptual and practical²⁴ implementation of this concept is the most critical element in current approval process. In the view of this expert committee the concept was clearly rooted in the existing paradigm for new crop development through traditional methodologies. A breeder who has genetically manipulated a crop through crossing/selection takes it as a given that, despite the numerous small changes introduced into the genome of the new genotype, the species as an entity remains largely unmodified. The expert panel however argued that present framing of the concept of substantial equivalence links it intimately with the definition of 'novel trait' in a way that leads to a logical impasse. This logical confusion has led to an ambiguous use of 'substantial equivalence' in the regulatory world. To the opinion of the expert committee, there were now two different kinds of the use of this concept: the *decision threshold* interpretation and the *safety standard* interpretation. The expert committee recommended the latter. "A GM organism is substantial equivalent if rigorous scientific analysis establishes that, despite all changes introduced into the organism as a result of the introduction of novel genes, the organism poses no more risk to health or to the environment than does its conventional counterpart."

Under the heading "Can substantial equivalence become scientifically rigorous?" the expert panel therefore suggested an integrated approach at six relevant levels (genome, transcript, protein, metabolite, health impacts, environmental impacts). The answers obtained from the molecular analyses, in particular at levels 1 to 4, would speak directly to the validity of the simple linear model of 'precise' genetic engineering. If these analyses are conducted on a range of existing transgenic varieties and the predictions of the simple linear model prove to be valid, that outcome would provide essential scientific support for the current regulatory view that the insertion of the transgene(s) has created no significant changes in the original variety other than those predicted and desired. If, on the other hand, the molecular analyses demonstrate that the simple model is not valid, the data would provide immediate entry points for studying the impacts of the detected changes on human health and the environment. The outcome of those follow-up studies will then help determine whether the impacts create a significant risk.

or other relevant parameters from conventionally bred plant varieties currently on the market. In the case of MON810 some statistically significant difference in composition have been observed but these changes were considered to be negligible. (See <http://www.cfsan.fda.gov/~lrd/biocon.html>).

²³ Elements of Precaution: Recommendations for the Regulation of Food Biotechnology in Canada, An Expert Panel Report prepared by the Royal Society of Canada, at the request of Health Canada, Canadian Food Inspection Agency and Environment Canada, February 2001.

²⁴ See for critical review : Clark, E.A. and Lehman, H., Assessment of GM Crops in Commercial Agriculture, Journal of Agricultural and Environmental Ethics, Vol. 13, No. 3-4, pg. 273-291, 2000. For example, one part of a submission of a GM potato to Canadian regulators involved 5 lines of Colorado potato beetle (CBP) resistant potato grown at four sites for one year, with six measured parameters (total solids, dextrose, sucrose, vitamin C, protein, glycoalkaloids). Twelve to fifteen replicated plots were sown per line at each site, but only four plots per site (total of 16 measured plots per line) were analysed. The results were accepted as evidence of substantial equivalence in nutritional composition. Differences between CBP lines and the parental control with regard to contents of total solids, dextrose, sucrose, vitamin C, protein, glycoalkaloids ranged from 0 to 25% depending on the line. Yet all 5 lines were found to be substantially equivalent. Because all GM submissions to date, in both the US and Canada, have been found to be substantially equivalent, it is unclear in the view of Clark how different something would have to be in order for it not to be found substantially equivalent.

ANNEX II

SUBSTANTIAL EQUIVALENCE AND EUROPEAN LEGISLATION

European Community Regulation 258/97, January 1997

The European Community Regulation²⁵ (EC) 258/97 on Novel Foods and Food ingredients sets out rules for notification or authorisation and labelling of GMO-derived food products and other novel foods.

Authorisation

In general²⁶ the authorisation for food use of a GMO-derived product is a one-step process if all Members States agree to the initial assessment of a Member State, and a two-step process if one or more Member States object. The first step is an initial assessment by the Member State where the food is to be placed on the market first. In case of a favourable opinion, this Member States informs the other Member States via the Commission. If there are no objections against the application, the first Member State can authorise for the whole Community and the product can circulate freely in the Single Market. This procedure can be completed within several months. If there are objections by Member states, the second step has to be taken, which means a Commission Decision, following consultation of the Scientific Committees, such as the Scientific Committee in Foods (SCF) and the Scientific Committee on Plants (SCP) on matters relating to public and the Comitology procedure. Once at community level, the time frame for authorisation is necessarily extended.

Notification

As a derogation²⁷ from the full authorisation procedure, the Novel Foods Regulation provides for a simplified procedure for foods derived from GMOs but no longer containing GMOs which are "substantially equivalent" to existing foods with respect to composition, nutritional value, metabolism, intended use and the level of undesirable substances. In such cases the companies only have to notify the Commission when placing a product on the market together with either scientific justification that the product is substantially equivalent or an opinion to the same effect delivered by the competent authorities of a Member State. The product can then be marketed in the entire EU. It should however be noted that in all cases of notifications analysed in this report, the SCP and/or SCF have also issued an opinion on whether the (ingredients of) GM food crops were to be viewed as substantial equivalent (or not). Both scientific committees fall under the European Commission Directorate General Health and Consumer Protection.

Noteworthy is the role of SCP as it has also delivered opinions on the risk assessment of these GM food crops as required by Directive 90/200 regulating experimental and commercial releases into the environment of GM plants, for which the European Commission Directorate General Environment is the main responsible body.

The following table shows examples of GM crops or ingredients derived thereof, which have been notified (or authorised) for food use in the European Union until May 2001. In practice mainly the UK, Germany and The Netherlands have received requests for notification or authorisation of GM food crops.

²⁵ Regulation (EC) of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients, Official Journal of the European Communities, L 43, Vol. 40, 14 February 1997.

²⁶ European Commission, Facts on GMOS in the EU, MEMO/00/43, Brussels, 13 July 2000.

²⁷ Article 3.4 Regulation 258/97.

GM FOOD CROPS AND SUBSTANTIAL EQUIVALENCE IN THE EU

Examples of (ingredients derived of) GM oilseed rape and GM maize notified (or authorised) for food use in the EU

Applicant	Food ingredient	Initial assessment	Assessment By others	Notification	Transmission to Member States	Evaluated in this report
AgrEvo (Aventis)	Refined oil from glufosinate-R oilseed rape TOPAS 19/2 and all conventional crossed	ACNFP 06.01.1995	SCP 10.02.1998	09.06.1997	24.06.1997	Yes
PGS (Aventis)	Refined oil from glufosinate-R hybrid oilseed rape MS1xRF1, MS1xRF2	ACNFP 19.09.1995		10.06.1997	24.06.1997 28.07.1998 again	No
Monsanto	Refined oil from glyphosate-R oilseed rape GT73	ACNFP 24.01.1996	VNV outside EU procedure	10.11.97	21.10.1997	No
Monsanto	Products from Bt-maize MON810	ACNFP 14.02.97	SCP 10.02.1998	10.12.1997	06.02.1998	Yes
AgrEvo (Aventis)	Products from glufosinate-R maize T25	ACNFP 14.02.97	SCP 10.02.1998 Comments Italy SCF 07.09.2000	12.01.1998	06.02.1998	Yes
Pioneer	Products from crosses of T25 and MON810	VNV Pending	SCP 06.06.2000	Request for authorisation		Yes
Novartis (Syngenta)	Products from Bt <i>silage</i> maize Bt11	ACNFP 14.02.97	SCP 10.02.1998 Comments Italy SCF 07.09.2000 SCP 30.11.2000	30.01.1998	06.02.1998	Yes
Novartis (Syngenta)	Products from Bt <i>sweet</i> maize Bt11	VNV 27.04.00		Request for authorisation		Yes
Monsanto	Products from glyphosate-R maize GA21	VNV 21.12.99		Request for authorisation		Yes
Pioneer	Products from glyphosate-R and Bt-maize MON809	ACNFP 14.02.97	SCP 19.05.1998 Comments Italy SCF 07.09.2000	14.10.1998	23.10.1998	No
AgrEvo (Aventis)	Refined oil from glufosinate-R oilseed rape LIBERATOR L 62 Phoe 6/Ac	BgVV	EU SCP 30.11.2000	21.10.1999	8/9.11.1999	Yes
AgrEvo (Aventis)	Refined oil from glufosinate-R oilseed rape Falcon GS 40/90	BgVV	SCP 14.07.2000	21.10.1999	8/9.11.1999	Yes
PGS (Aventis)	Refined oil from glufosinate-R hybrid oilseed rape MS8xRF3	BgVV	SCP 19.05.1998	21.10.1999	8/9.11.1999	Yes

- ACNFP: Advisory Committee on Novel Foods and Processes (United Kingdom); BgVV: Bundesamt für gesundheitliche Verbraucherschutz und Veterinärmedizin (Germany); VNV: Veiligheidscommissie Nieuwe Voedingsmiddelen (The Netherlands).

Evaluation of experience gained

Paragraph 1 of Article 14 of Regulation 258/97 stipulates that no later than five years from the date of entry into force and in the light of the experience gained, the Commission shall forward to the European Parliament and to the Council a report on its implementation. The date of entry was 27 January 1997. This implies that the Commission should forward this report at the latest on 27 January 2002.

EC Scientific Committee for Food, July 1997

As part of the development of Regulation 258/97 the European Commission asked the Scientific Committee for Food (SCF) to develop recommendations²⁸ concerning the scientific aspects necessary to support an application on the market of novel foods and novel food ingredients. With reference to OECD and WHO the SCF reminded that establishment of substantial equivalence is not a safety or nutritional assessment in itself but an approach to compare a novel food with its conventional counterpart.

Substantial equivalence may be established either for the whole food or food component including the introduced 'new' change, or it might be established for the food or food component except for the 'new' change introduced. If substantial equivalence of a novel food or food component has been established, it means that their safety is comparable to that of their conventional counterparts. If a novel food has not been found to be substantially equivalent, this does not imply that it is unsafe. It just indicates that it should be evaluated on the basis of its unique composition and properties.

In the view of the SCF, analytical studies are of crucial importance not only for the establishment of substantial equivalence but also as a prerequisite for nutritional and toxicological assessments. Methods applied have to be standardised and validated to ensure quality and consistency of data. Investigations should especially focus on the content of critical macro- and micro-nutrients, any critical toxicants and anti-nutritional factors, which might be either inherently present or due to the modification.

EC Scientific Committee on Plants, December 1998

On 18 December 1998 the Scientific Committee on Plants (SCP) of the European Commission published a guidance document²⁹ to facilitate applicants in the preparation of GM plant dossiers.

With respect to the establishment of substantial equivalence, SCP recommended that data should be obtained from a valid comparison of GM plants and a conventional line, which is preferably isogenic, based on samples from at least two seasons, grown at a number and variety of geographical locations. The data should further be accompanied by an appropriate statistical treatment. The specific analyses would depend on the plant species examined. Applicants were also encouraged to provide data, which demonstrate the range in component concentrations, found in non-GM counterparts and thereby to make comparisons with the GM plant in question.

²⁸ Commission recommendations of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under regulation (EC) No 258/97 of the European Parliament and Council, Official Journal of the European Communities, L 253, Vol. 40, 16 September 1997.

²⁹ Guidance document to facilitate notifiers in the preparation of plant GMO dossiers for consideration by the Scientific Committee on Plants (SCP/GMO/103-final) – Opinion expressed on 18 December 1998.

UK Food Standards Agency, considerations for statistically valid data, May 1999

In 1999 the UK competent authority, the Food Standards Agency, presented to the European Commission a paper³⁰ to promote discussion about the provision of statistically valid data in applications for products approvals under the EC Novel Foods Regulation. The paper resulted from work by the UK Advisory Committee on Novel Foods and Process (ACNFP).

As starting point this paper took the 1996 FAO/WHO expert consultation statement that “substantial equivalence is established by a demonstration that the characteristics assessed for the GMO, or the specific food product thereof, are equivalent to the same characteristics of the conventional comparator. The levels and variation of characteristics (e.g. phenotypic and composition characteristics etc.) in the GMO must be within the natural range of variation for those characteristics considered in the comparator and be based upon an appropriate analysis of data”.

The paper then detailed the factors, which might affect ‘natural variation’, the confidence limits and sample size, and the number of trial sites, years and replicates. It concluded that statistically valid data are necessary for effective comparison of a GM crop with a non-GM control. The use of such data would minimise the effects of natural variation and would indicate whether there are any differences between the GM crop and the non-GM crop, which require further investigation. Significant difference between the results of proximate and detailed chemical analyses of a GM crop product and its non-GM control may be an indication of secondary or pleiotropic effects. It further recommended that trials with GM crops be conducted under the range of environmental conditions in which it is intended that the GM crop should be grown commercially. A minimum of six sites should be used, with the number of replicates to be determined for individual crop species, and trials should be carried out for a minimum of two successive years. The paper further recommended that the Commission seek EU-wide agreement on an appropriate confidence interval to use in the statistical analyses and the sample size. The paper noted that 95% confidence intervals are commonly used. In addition, data from different sites should not be pooled before being statistically evaluated.

EC Scientific Steering Committee, October 2000

With reference to the FAO/WHO Consultation of May 2000, the European Commission Scientific Steering Committee³¹ (SSC) was due to the opinion that the establishment of substantial equivalence should be used as a starting point to guide the safety evaluation. It noted that an operational definition was needed as the various European Commission scientific committees had applied the concept on a case by case basis. It proposed that this issue should be the focus of a future opinion.

In the view of the SSC, the routine analytical procedures in use still needed to be standardised in terms of sampling and extraction procedures, validation of profiling methods and in bio-informatics. Especially if transgenic material is introduced by gene technology³² or

³⁰ Paper for consideration by the European Commission, Statistically valid data to support applications for safety clearance of crop products under EC Regulation on Novel Foods and Ingredients 258/97, 19 May 1999, <http://www.foodstandards.gov.uk/maff>. (See also ACNFP 1998 Annual Report, Annex VIII.)

³¹ Risk assessment in a rapidly evolving field: the case of GM plants, Scientific Opinion of the Scientific Steering Committee, European Commission, Expressed on 26/27 October 2000.

³² Kleter, G.A., et al, New developments in crop plant biotechnology and their possible implications for food product safety, Literature study under commission of the Foundation “Consument en Biotechnologie”, RIKILT, Wageningen University Research, Report 2000.004, March 2000: “This will render the assessment of substantial equivalence more complex. In crops with multiple genes, all genes will have to be assessed individually and theoretically chances of secondary effects will increase. Further, the nutritional value and wholesomeness of the modified crop may be affected. It will therefore be important to improve risk assessment strategies, especially if in the future more varieties are

by modern conventional plant breeding to alter an existing metabolic pathway or introduce a new one, toxicants might accumulate. The methods applied did not provide certainty to detect all new non-targeted toxic plant constituents or an increase of the amount of unidentified existing toxic constituents. The methods therefore, needed to be expanded. The SSC expected that tools (under development)³³ such as genomics, proteomics and metabolomics would offer greater opportunities for their food safety evaluation.

Scientific review of substantial equivalence assessments, January 2000

In a review article³⁴ on the potential pleiotropic effects of genetic modifications on constituents of modified crops data from US and EC documents were investigated with regard to inherent plant toxins and anti-nutrients: rape (glucosinolates, phytate), maize (phytate), tomato (tomatine, solanine, chaconine, lectins, oxalate), potato (solanine, chaconine, protease inhibitors, phenols) and soybean (protease-inhibitors, lectins, isoflavones, phytate). According to the authors, genetic modification of food-plants is developing rapidly, but until now no long-term experience on nutritional (or ecological) effects is available. In the view of the authors, especially the relevance of pleiotropic effects due to the genetic modification is unclear, but could play a role in the question of possible changes in the expression of constituents such as inherent plant toxins and anti-nutrients. The impossibility of a prediction of the integration region of the genetic modification is one of the main problems for the estimation of the probability and of the dimension of pleiotropic effects. The authors recognised that the concept of substantial equivalence had been seriously criticised (Millstone et al., 1999)³⁵ but strong scientific support had defended the use of this concept. Although an assessment of substantial equivalence is necessary for notification in the EU, the authors' analysis of selected notification documents of GM plants indicated that relevant data about inherent plant toxins and anti-nutrients were often missing. Some documents provided no data at all about inherent plant toxins and anti-nutrients, and in a few cases applicants argued that analysis of some constituents was not relevant. In fact, there were no coherent regulations to which companies could adhere for the selection of inherent plant toxins and anti-nutrients that have to be analysed. The documents showed, in the view of the authors, no consistency. The assessments of substantial equivalence could therefore hardly be conclusive. Levels of inherent plant toxins and anti-nutrients were found within the range of the non-transformed parental plants in most GM plants. However, in several documents no or incomplete data on these compounds could be found, for example with regard to saponins in tomato (Zeneca, 1996) or phenolic compounds and coumarins in potato (Avebe, 1996). In the dossier of GM tomato many anti-nutrients were tested, such as glycoalkaloids and lectins, but no data were provided about oxalate (Zeneca, 1996). In addition, only in one of the four notification of GM maize varieties phytate levels were analysed. Since, according to the authors, phytate occurs in considerable amounts in maize, its levels should be analysed for an assessment of the substantial equivalence of the GM plant. The silage and grain of glufosinate-tolerant maize (AgrEvo, 1995) was found not to be different from commercial varieties in essential nutrients or inherent plant toxins and anti-nutrients. In preliminary documents of the GM glyphosate-tolerant maize line GA21 (Monsanto 1998), compositional components, such as protein, fat, ash, carbohydrates, moisture and fibre, amino acid composition and fatty acid profile, calcium

marketed with changed nutritional characteristics. In order to assess actual intake levels in the different consumer groups, it will be necessary to further improve the traceability in well-documented production chains. Additional insight should be gained into consumption patterns within the EU and abroad in general and with respect to specific consumer groups such as small children and the elderly."

³³ In Europe a research consortium ENTRANSFOOD funded by the EC Directorate-General Research Framework Programme V has recently been initiated to further develop these tools and to assess their relevance to demonstrating substantial equivalence and unintended effects. (This research work in progress is accessible at <http://www.entransfood.org>.)

³⁴ Kovak, W., K. and Haslberger, A.G., Substantial equivalence of Antinutrients and Inherent Plant Toxins in Genetically Modified Novel Foods, Food and Chemical Toxicology, Vol. 38, pg. 473-483, 2000.

³⁵ See footnote 6.

and phosphorus were analysed, but no inherent plant toxins and anti-nutrients, such as phytate, were included in the analyses.

Further, in one dossier on modified rape plants (Plants Genetic Systems, 1996) the values of glucosinolates in the modified plant were significantly different (higher) than in the non-transformed plants, and in some cases significant differences between the glucosinolate content of one modified line and its non-transgenic counterpart were observed. It was noticed that the glucosinolate content varied more between the different locations than between the different transgenic and non-transgenic entries in a given location. These variations were discussed by the applicant, suggesting it is generally accepted that commercial food derived of plants exhibits considerable variability in its compositions and that this variability is more the result of the interaction of the genotype with the environment, rather than the results of the insertion of specific genes into the plant genome. But, in the view of the authors, this conjecture may not be scientifically justified: how much is the accepted range of the variability, what are the regulations for variations above the statistical limits, what variations may be dangerous? These questions were not completely clarified yet. The amounts and natural variation of anti-nutrients in one crop plant species could differ considerably due to factors such as state of ripening, year of production, storage, varietal differences, growing conditions (climate, soil) and stress or pathogen infection. Literature data sometime showed very wide variations in typical plant constituents. And although ranges for most of the compositional variables were available in literature, these data might not be directly comparable due to differences in analytical methods or sample preparation. In addition, much of the literature data were relatively old and might not encompass the compositional variables of modern crop varieties. Comparisons between studies were often further complicated because parameters of analysis like units and basis of standardisation of substances showed no conformity and were difficult to interpret.

In conclusion, the authors argued that their review supported the usefulness of the concept of substantial equivalence. But they viewed the lack of specification as to which key components have to be analysed in a certain GM plant to establish substantial equivalence as a major problem. A minimum list³⁶ of macro- and micro-nutrients, secondary plant constituents, inherent plant toxins and anti-nutrients and allergens known to be associated with a crop species that should be analysed in order to determine substantial equivalence should be established. Another difficult problem for determining substantial equivalence was that it is not always technically possible to use authentic isogenic control lines. The process to obtain all the required permissions takes a long time, and in the end the compared data from transgenic lines often come from very early transformants and are not really consistent with the final products. In addition, special care has to be taken in investigating and controlling possible effects of environmental conditions on constituents of GM crops. Although such effects, possibly caused by unusual environmental parameters, could similarly be seen with conventional crops, several problems to agronomic characteristics have been observed³⁷ in GM crops due to unusual temperatures and possible changes in plant physiology caused by the insertion of genes. In the view of the authors, such observations, together with a specific public awareness, suggest that conclusive information requirements for notifications and a scientifically reviewed, publicly available risk assessments, as well as post-marketing controls would be important to safely use gene technology in food and feed production.

³⁶ References provided by the authors: 1) Nordic Council of Ministers, Safety Assessment of Novel Foods Plants, Chemical analytical approaches to the Establishment of Substantial Equivalence, 591, Tema Nord, Copenhagen, 1998, and 2) so-called 'OECD consensus documents' from Series on Harmonization of Regulatory Oversight in Biotechnology. For example, Consensus Document No. 7 concerns oil seed rape (1997); this document however does not provide information relevant to establish substantial equivalence.

³⁷ References provided by the authors: 1) Fox, J.L., Bt cotton infestations renew resistance concerns, Nature BioTechnology, Vol. 14, pg. 1070-1074, 1996, and 2) Kaiser, J., Pests overwhelm Bt cotton crop, Science, vol. 273, pg. 423-427, 1996.

Chemical fingerprinting of unintended secondary metabolic changes, 2000

One of the key issues in the safety assessment of GM food crops is to determine whether 'unintended' effects may have occurred as a result of the genetic modification. However, in the view of Noteborn³⁸ (2000), a compositional analysis based on single compounds has its limitations with respect to (unknown) anti-nutrients and natural toxicants. Novel technologies and detailed analyses of the (food) foreign gene products and of possible 'unintended' effects in the plant were therefore urgently needed. In the study the utility of a combination of nuclear magnetic resonance (NMR) spectroscopy and liquid chromatography (LC) for the multi-component comparison of low-molecular compounds (i.e. chemical fingerprinting) in complex plant matrices was demonstrated and discussed. This methodology might further contribute to the demonstration of substantial equivalence by its ability to compare compositional changes with regard to a couple of hundred low-molecular compounds between a GM food crop and related non-GM reference lines. The study used two different kinds of GM tomatoes as model crops: a GM tomato with a down-regulated expression of exogalactanase leading to altered texture, mouthfeel and colour and a GM tomato expressing a *Bacillus thuringiensis* (*cry1Ab5*) gene conveying resistance to *Lepidoptera* pests.

The study also resulted in a proposal for a hierarchical approach by comparing the chemical fingerprints of the GM plant to those of: 1) isogenic parental or closely related lines bred at identical and multiple sites; 2) extended ranges of commercial varieties of that plant; and 3) downstream processing effects. In the view of the authors, this would be important to assess the likelihood that the statistical differences in a GM crop plant might be false positives due to chance alone, or arose from natural genetic and/or physiological variations.

EC Scientific Committee on Plants, April 2001

In April 2001, under the remit of the Scientific Committee on Foods (SCF), the Scientific Committee on Animal Nutrition (SCAN), and the Scientific Committee on Plants (SCP)³⁹, a SCP working group of experts started to draft a guidance document for applicants, who are seeking authorisation for a GMO release and subsequently authorisation for their food and use. This guidance document should in principle be adopted by the Scientific Steering Committee (SSC). It would provide guidance for the molecular characterisation of the gene construct, the establishment of substantial equivalence, performing the safety assessment, the nutritional assessment and the environmental assessment.

³⁸ Noteborn, H.P.J.M., Lommen, A., Van der Jagt, R.C., Weseman, J.M., Chemical fingerprinting for the evaluation of unintended secondary metabolic changes in transgenic food crops, *Journal of Biotechnology* 77, pg. 103 – 114, 2000.

³⁹ See http://europa.eu.int/comm/food/fs/sc/scp/out_en.html

ANNEX III**CASE STUDIES OF SUBSTANTIAL EQUIVALENCE ASSESSMENTS IN THE EU**

III.1 REFINED OIL OF TRANSFORMATION EVENT LIBERATOR PHOE6/AC

Plant:	Swede rape
Main trait of interest:	Glufosinate-tolerance
Applicant:	AgrEvo
Directive 90/220 nr.:	C/DE/98/6 (uses as any other swede rape, received by Commission on 29.10.1998, pending)
Regulation 258/97:	Notification transmitted to member states on 8/9.11.1999

Available documentation and information

1. Application file C/DE/98/6.
2. Refined oil derived of Liberator pHoe6/Ac notified by Germany on 21 October 1999. Correspondence between BgVV, the European Commission Enterprise Directorate-General and the Dutch Committee Safety Evaluation of Novel Foods and personal communication with Mrs Schauzu of BgVV on May 3rd, 2001.
3. Additional information from Aventis on 19 June 2000.
4. Opinion of Scientific Committee on Plants issued on 30 November 2000.

III.1.1 Application file C/DE/98/6

The applicant submitted data (minimum and maximum values) on the contents of oil, ash, crude fibre, sinapine, phytate, glucosinolates and amino acids in desolventising meal of GM oilseed rape plants, control-plants and commercial varieties. Data on levels of chlorophyll, tocopherols, sterols, P, Fe, Cu, Ca, Mg, S, erucic and fatty acids in blending crude oil and deodorised oil were also provided. Samples were pooled from the harvest of two different locations in Canada in 1997. Although the data were not statistically analysed, the applicant concluded that no changes in most of the important compounds in the GM oilseed rape plants compared to its controls had occurred.

III.1.2 BgVV, Germany, 30 September 1999

BgVV's initial assessment is based on semi-quantitative data on the contents of protein, oil and glucosinolates and on qualitative and quantitative data on amino acid composition of the flour, fatty acid composition of the seed and oil, of which also the contents of sterols and tocopherols have been analysed. Protein content of refined oil of Liberator pHoe6/Ac has been determined at 0.01 mg/ml based on data submitted in the case other GM oilseed rape lines, i.e. Topas 19/2 and MS8xRF3. According to BgVV, refined oil of Liberator pHoe6/Ac is substantial equivalent to that of its comparator, the parental line Liberator regarding nutritional value, intended use, composition and the level of undesirable substances. On 21 October 1999 the applicant notified the refined oil of Liberator pHoe6/Ac to the European Commission, which subsequently informed the other member states about this notification on 8 November 1999.

The correspondence between the Commission and the member states shows that a few member states noted that the data were obtained from winter oilseed rape varieties only, while consent for commercial release of transformation event Liberator pHoe6/Ac might also include spring oilseed rape varieties based on this transformation event. Further, Denmark did not view this GM oilseed rape plant as substantial equivalent. It noticed from the data

submitted that in one of two lots the GM oilseed rape plants had a 50% higher erucic acid content than their unmodified counterparts, whereas in the other lot the GM oilseed rape plants showed a 35% decrease. Levels of tocopherols were 20 to 45% higher in the GM oilseed rape plants than in their counterparts. Chlorophyll content in the GM oilseed rape plants increased with 400% (which also led to questions by The Netherlands), and desolventising meal of the GM oilseed rape plants contained 20 to 25% more glucosinolates.

III.1.3 Additional information from Aventis, 19 June 2000

In response to questions raised by Italy, Portugal, United Kingdom and Ireland, Aventis submitted additional information confirming that no modified DNA or modified protein is present in refined oil. Aventis further provided technical information about the application of High Performance Liquid Chromatography (HPLC) to determine the content of glucosinolates in the oil of MS8xRF3, respectively Gas Liquid Chromatography (GLC) in the case of Falcon GS40/90 and Near Infrared Reflectance Spectroscopy (NIRS) in the case of Liberator pHoe6/Ac, indicating that these three different methods of analysis had been adequate in these cases.

III.1.4 EC Scientific Committee on Plants, 30 November 2000

COMPONENT	DATA/SOURCE	REMARKS
Oleic acid, erucic acid	Harvest of one year in UK	No conspicuous or consistent differences
Protein, oil, glucosinolates	Harvest of two years in Germany	No conspicuous or consistent differences
Fatty acids	Harvest of two years Germany	No differences
Macro-components, anti-nutrients, amino acids	Desolventised meal from harvest of one year in Canada	No conspicuous differences
Fatty acids, erucic acid	Blending crude oil from harvest of one year in Canada	No conspicuous differences
Chlorophyll, sterols, tocopherols	Blending crude oil from harvest of one year in Canada	No conspicuous differences apart from a higher level of chlorophyll for GM plants. This could have originated from contaminated green (instead of yellow) seeds; not considered relevant
Oil	Bleached and deodourised oil from harvest on one year in Canada	Equivalent

From the data provided, the SCP concluded that genetically modified oilseed rape varieties derived from Liberator pHoe6/Ac are substantially equivalent to their conventional counterparts except for the introduced traits.

III.2 REFINED OIL OF TRANSFORMATION EVENT FALCON GS40/90

Plant: Swede rape
Main trait of interest: Glufosinate-tolerance
Applicant: AgrEvo
Directive 90/220 nr.: C/DE/96/05 (uses as any other swede rape, received by Commission on 25.11.1996, pending)
Regulation 258/97: Notification transmitted to Member States on 8/9.11.1999

Available documentation and information

1. Opinion of Scientific Committee on Plants issued on 14 July 1998.
2. Application file C/DE/96/05.
3. Refined oil derived of Falcon GS40/90 notified by Germany on 21 October 1999. Correspondence between BgVV, the European Commission Enterprise Directorate-General and the Dutch Committee Safety Evaluation of Novel Foods and personal communication with Mrs Schauzu of BGVV on May 3rd, 2001.
4. Additional information from Aventis on 19 June 2000.

III.2.1 EC Scientific Committee on Plants, 14 July 1998

COMPONENT	DATA/SOURCE	REMARKS
Oil, crude fibre, ash, protein, fatty acids, glucosinolates, sterols, and tocopherols	Whole seed, seed meal, and extracted oil	No changes related to the process of genetic modification. In case of seed meal the observed difference in oil content are considered to be due to the production process

The SCP concluded that the GM plants may be considered substantial equivalent to their unmodified counterparts except for the inserted traits.

III.2.2 Application file C/DE/96/05

In its report (3 August 1999) on this application (originally submitted in Germany in 1996) to the Dutch advisory body COGEM (Committee on Genetic Modification) for Directive 90/220, the governmental research institute RIKILT noted that in first instance the composition of seeds from 8 plots with GM oilseed rape plants unsprayed and sprayed with glufosinate and the non-GM line had been analysed with respect to oil, protein, fatty acids, amino acids, dry matter and glucosinolates. Average levels of oil were slightly higher in the GM seeds than in non-GM seeds. In the view of RIKILT, the application file lacked: 1) a statistical analysis of the results, 2) data on minerals or ash, vitamins (especially vitamin E), 3) data on individual glucosinolates in seed and meal, and contents of sterols in oil. The applicant subsequently provided these data, which led to RIKILT's observation that the content of oil in the GM seeds was slightly lower than in non-GM seeds. The difference in seed was not consistent. In meal a larger difference in oil content was found. This might be due to the experimental processing of relatively small batches of GM seeds. The contents of individual glucosinolates in GM meal were lower than in non-GM meal in most cases. Nonetheless, in the view of RIKILT, the GM seeds did not differ substantially from non-GM seeds with the exception of the presence of introduced trait.

III.2.3 BgVV, Germany, 23 July 1999

BgVV's initial assessment is based on data on the contents of protein, oil, oleic acid, dry residue and glucosinolates and on qualitative and quantitative data on the contents of amino

acids and fatty acids. Protein content of refined oil of Falcon GS40/90 has been determined at 0.01 mg per ml based on data submitted in the case other GM oilseed rape lines, i.e. Topas 19/2 and MS8xRF3. According to BgVV, refined oil of Falcon GS40/90 is substantial equivalent to that of its comparator regarding nutritional value, intended use, composition and the level of undesirable substances.

On 21 October 1999 the applicant notified the refined oil of Falcon GS40/90 to the European Commission, which subsequently informed the other member states about this notification on 9 November 1999.

III.2.4 Additional information Aventis, 19 June 2000

In response to questions raised by Italy, Portugal, United Kingdom and Ireland, Aventis submitted additional information confirming that no modified DNA or modified protein is present in refined oil. Aventis further provided technical information about the application of High Performance Liquid Chromatography (HPLC) to determine the content of glucosinolates in the oil of MS8xRF3, respectively Gas Liquid Chromatography (GLC) in the case of Falcon GS40/90 and Near Infrared Reflectance Spectroscopy (NIRS) in the case of Liberator pHoe6/Ac, indicating that these three different methods of analysis had been adequate in these cases.

III.3 REFINED OIL OF TRANSFORMATION EVENT MS8XRF3

Plant: Swede rape
Main trait of interest: Hybridisation system
Applicant: Plant Genetic Systems
Directive 90/220 nr: C/B/96/01 (uses as any other swede rape, received by the Commission on 16.01.1997, pending)
Regulation 258/97: Notification transmitted to Member States on 8/9.11.1999

Available documentation and information

1. Application file C/B/96/01.
2. Opinion of Scientific Committee on Plants issued on 18 May 1998.
3. Refined oil derived of MS8xRF3 notified by Germany on 21 October 1999. Correspondence between BgVV, the European Commission Enterprise Directorate-General and the Dutch Committee Safety Evaluation of Novel Foods and personal communication with Mrs Schauzu of BGVV on May 3rd, 2001.
4. Additional information from Aventis on 19 June 2000.

III.3.1 Application file C/BE/96/01

On 7 January 1997 the Belgian Competent Authority for Directive 90/220 informed the European Commission and the other member states about its positive advice on the application. In this advice Belgium also established the substantial equivalence of this GM oilseed rape. This was based on data (minimum and maximum values) submitted by the applicant with respect to the contents of oil, fibre, vitamins, minerals, glucosinolates and amino acids in desolventising meal of GM oilseed rape plants, control-plants and commercial varieties. Data on levels of chlorophyll, tocopherols, sterols, P, Fe, Cu, Ca, Mg, S, erucic acid and fatty acids in blending crude oil and deodorised oil were also provided. Samples were pooled from the harvest of many locations in 1995. The data from the compositional analysis were not statistically analysed. In one case the applicant observed significant differences

between the content of glucosinolates of RF3 GM oilseed rape plants and of its unmodified counterpart due to environmental conditions.

III.3.2 EC Scientific Committee on Plants, 18 May 1998

COMPONENT	DATA/SOURCE	REMARKS
Oil, glucosinolates, fatty acids, erucic acid, vitamin E and minerals	Seeds harvested from original plant lines, their hybrids, and lines developed from them by multiple backcrosses to different oilseed rape varieties from field trials in Europe and North America; locally adapted varieties were used as controls	Ranges for all GM lines fell within the range for the non-modified lines grown in each trial.

III.3.3 BgVV, Germany, 30 September 1999

BgVV's initial assessment was based on a comparative analysis of the composition of transgenic and control non-transgenic seeds regarding total protein, oil, glucosinolates, vitamin E, phytic acid, fibre, moisture and dry residues and on qualitative and quantitative data regarding amino acid and fatty acid composition. According to BgVV, refined oil of MS8xRF3 is substantial equivalent to that of its comparator regarding nutritional value, intended use, composition and the level of undesirable substances

On 21 October 1999 the applicant notified the refined oil of MS8xRF3 to the European Commission, which subsequently informed the other member states about this notification on 9 November 1999.

III.3.4 Additional information Aventis, 19 June 2000

In response to questions raised by Italy, Portugal, United Kingdom and Ireland, Aventis submitted additional information confirming that no modified DNA or modified protein is present in refined oil. Aventis further provided technical information about the application of High Performance Liquid Chromatography (HPLC) to determine the content of glucosinolates in the oil of MS8xRF3, respectively Gas Liquid Chromatography (GLC) in the case of Falcon GS40/90 and Near Infrared Reflectance Spectroscopy (NIRS) in the case of Liberator pHoe6/Ac, indicating that these three different methods of analysis had been adequate in these cases.

III.4 REFINED OIL OF TRANSFORMATION EVENT TOPAS 19/2

Plant: Rape
Main trait of interest: Glufosinate-tolerance
Applicant: AgrEvo
Directive 90/220 nr.: C/UK/95/M5/1 (import and processing, approved 22.04.1998)
Regulation 258/97: Notification transmitted to Member States on 24.06.1997

Available documentation and information

1. Refined oil derived of Topas 19/2 notified by United Kingdom on 9 June 1997.
2. Opinion of Scientific Committee on Plants issued on 10 February 1998.

III.4.1 ACNFP, United Kingdom, 9 June 1997

Based on compositional analysis of the contents of oil, fatty acids, erucic acid and glucosinolates in samples from trials at a number of locations in several, successive years, the UK Advisory Committee Novel Foods and Processes ACNFP concluded that no unintentional changes or pleiotropic effects had occurred. It was further demonstrated that no modified DNA or protein was present in refined oil of Topas 19/2. The UK authority concluded that the refined oil did not differ in composition from conventional oilseed rape and was therefore as safe as oil of conventionally bred oilseed rape. However, as there was little experience in predicting the effect of genetic drift on the metabolism of any line of plants, whether GM or non-GM, the UK authority asked the applicant to monitor the seed composition and the fatty acid profile of GM swede rape lines over time.

III.4.2 EC Scientific Committee on Plants, 10 February 1998

COMPONENTS	DATA/SOURCE	REMARKS
Oil, fatty acids, erucic acid, glucosinolates	Seed harvested from trials at a number of locations within Canada in several successive years	Data for the seed from GM plants fall within the range for non-GM control varieties. For food purposes the product is likely to be highly processed so that both the genetic material introduced into the transgenic plant and its protein products would be absent from the refined product.

On the basis of substantial equivalence, the SCP concluded that refined products from plants derived from this glufosinate-tolerant plant would be safe for food use.

III.5 PROCESSED PRODUCTS FROM TRANSFORMATION EVENT BT11 MAIZE

Plant: Maize
Main trait of interest: Insect-resistance and glufosinate-tolerance
Applicant: Novartis
Directive 90/220 nr.: C/UK/96/M4/1 (import and processing, approved 22.04.1998)
C/F/96/05-10 (cultivation, received by the Commission on 12.04.1999, pending)
C/ES/98/02 (cultivation, received by the Commission on 03.05.1999, pending)
Regulation 258/97: Notification Bt11 silage maize transmitted to Member States on 06.02.1998
Request for authorisation Bt sweet maize pending;
initial assessment by The Netherlands on 27.04.2000

Available documentation and information

1. Initial assessment of Bt11 *silage* maize by ACNFP, United Kingdom, 14 February 1997.
2. Opinion of Scientific Committee on Plants, 10 February 1998.
3. Request for authorisation of fresh and processed kernels of Bt11 *sweet* maize submitted in The Netherlands. Initial assessment completed 27 April 2000. Questions on initial assessment raised by member states and response of Syngenta from 29 November 2000.

4. Opinion of the Scientific Committee on Food concerning a submission from the Italian authorities raising concerns for the safety of certain products approved under the notification procedure of Regulation (EC) 258/97, 7 September 2000.
5. Opinion of Scientific Committee on Plants, 30 November 2000.

III.5.1 ACNFP, United Kingdom, 14 February 1997: Bt11 silage maize

The applicant submitted data from the compositional analyses on grain harvested from four hybrid lines derived of BT11 and four conventionally bred hybrid lines with the same genetic background but lacking the introduced genes. These lines were chosen to represent middle and late maturity groups. Contents of starch, protein, oil and fibre were analysed using near infrared spectroscopy. Statistically significant differences were found between the middle maturity GM hybrids and the corresponding conventionally bred lines for protein content, resulting of the conventional backcross breeding carried out. Values for all the parameters measured fell within the ranges cited in published literature. Fatty acid and amino acid analyses were performed on twenty samples of grains. Apart from normal variations, there were no significant differences in composition. ACNFP concluded that GM maize lines derived from B11 would not differ in composition from those from conventionally bred maize.

However, there was little experience in predicting the effect of genetic drift on the metabolism of any kind of plants, whether genetically modified or conventionally bred. ACNFP therefore asked the applicant that to monitor the seed composition, including fatty acid and amino acid profiles of the oil derived of Bt11 hybrids and the composition of the grains over time.

III.5.2 EC Scientific Committee on Plants, 10 February 1998

Compositional analysis of seeds harvested from trials at a number of locations within the US provided data on oil content, fatty acid composition, fibre and starch content and amino acid profiles. The composition of the GM plants fell within the range observed for non-GM plants and isogenic control varieties. On the basis of substantial equivalence SCP concluded that grain or products derived from imported grain harbouring Bt11 event would be safe for food use.

III.5.3 VNV, The Netherlands, 27 April 2000: Bt11 sweet maize

The request for a full safety evaluation of products of Bt11 sweet maize was submitted by Novartis to The Netherlands in April 1998. On 12 August 1999 the Committee Safety Evaluation of Novel Foods (Dutch acronym VNV) of the Health Council informed the applicant that to underpin the substantial equivalence of genetically modified and conventional maize kernels not only data on macro- and micro-nutrients are of importance but also data on secondary plant metabolites. VNV therefore requested the applicant to provide additional information regarding potential changes of the contents of secondary plant metabolites due to the genetic modification. The applicant subsequently conducted analyses to determine the content of furfural, p-coumaric acid, ferulin acid, raffinose and phytic acid and provided the information requested on 31 January 2000. On 29 November 2000 Novartis also responded to questions raised by several EU member states on the initial assessment by The Netherlands. According to the committee's advice on 27 April 2000, the applicant clearly followed the flowcharts annexed to Regulation 258/97 and the molecular-biological, nutritional and toxicological data provided were adequate for an evaluation by the committee.

III.5.3.1 Information on nutritional value

Maize kernels consist of starch (71%), protein (10%), oil (4.5%) and fibre (3%).⁴⁰ Four different studies have been conducted for compositional analysis of Bt11 maize plants and control plants. In the first study three Bt11 hybrids and their isogenic controls from field trials in California in 1996 had been compared concerning their content of moisture, protein, oil, ash, sugars, carbohydrates, vitamin A, vitamin C, and Na, K, Ca and Fe. The applicant did not argue why a statistical analysis had not been performed, whereas the committee viewed that such a statistical analysis should be done. In this case, looking at the figures in the tables, it was clear to VNV that the composition of GM maize and conventional maize only marginally differed regarding the components reported. The applicant did also not argue why the contents of P, S, Cl, I, F, Mn, Cu, Cd, Chr, Se, Co and Me had not been investigated, especially as P and S occur at relatively high levels in maize. The committee would like to learn the arguments why the micro-nutrients analysed had been selected to be analysed. Nonetheless the committee considered the data submitted adequate for an evaluation.

In the second study the composition of homozygous Bt11 maize plants and heterozygous Bt11 maize plants with their respective controls grown in greenhouses had been compared regarding their content of moisture, nitrogen, ash, starch, cellulose, xanthophyll and several fatty acids and amino acids. A difference of 0.6% between homozygous Bt11 maize and its control concerning nitrogen content had been found. To the opinion of the committee, this value was on the edge. All maize plants had high nitrogen levels probably due to application of artificial fertiliser in greenhouse cultivation.

In the third study kernel size, density, starch, protein, oil, fatty acids and amino acids of two Bt11 hybrids and their controls on three field locations had been analysed by infrared analysis. No relevant differences have been established apart from a significant lower level of protein in one Bt11 hybrid, but the value fell within range of values from literature and was therefore not considered as problematic by the committee. Significant differences (threshold of 5%) had been found for palmitic acid (higher in Bt11), stearic acid, cystine and arginine (higher in control). But since the differences between conventional lines are larger, the applicant did not view the differences relevant. The committee shared this viewpoint.

The fourth study concerned minerals and vitamins. The data for each Bt11 hybrid and their respective controls had been averaged over three locations and no significant difference had been found. The committee expressed the view that it would be more appropriate to provide such data per location and asked the applicant to submit these. It is however not clear whether such data have been provided.

III.5.3.2 Toxicological information

The *degree* of substantial equivalence of two Bt11 hybrids and the conventional parent line had been underpinned by a comparison of the composition from a nutritional perspective and by a further analysis of five secondary plant metabolites, e.g. furfural, raffinose, myo-inositol, p-coumarin acid and ferulin acid. Samples had been taken from three Bt11 hybrids and their respective controls grown at one location. No statistically significant differences had been found.

III.5.3.3 Questions raised by other EU member states

Austria indicated that sweet maize and silage maize have a different composition, so that it was not sufficient only to submit data on Bt11 silage maize. Further Austria, Germany and Italy questioned the statistical validity of analyses comparing the composition of Bt11 maize and its control. Austria felt that also data on the content of vitamin B, tocopherols, anti-

⁴⁰ Watson, S.A., Structure and composition. In: Watson, S.A. and Ramstad, R.E. (Eds.), Corn chemistry and technology, St Paul, Minnesota: Am Soc Cereal Chem, 1987: 3-29.

nutrients such as phytic acid and of minerals and trace elements like P and S should have been provided. The data submitted were therefore insufficient to establish substantial equivalence. Italy was due to the opinion that the criteria for selecting the micro-nutrients to be analysed should be indicated and that it is not appropriate to draw samples from maize plants grown in greenhouses. Portugal also expressed doubts regarding the criteria for the choice of analyses and regarding the variation in composition and therefore asked why P, S, Cl, I, Zn, F, Mn, Cu, Pb, Cd, Cr, Se, Co and Hg had not been analysed. Sweden indicated that the documentation on substantial equivalence should include data on vitamins and minerals. Germany noted that the nutritional composition had been determined on the basis of only one year's harvest and yearly variations could therefore not be taken into account. Germany also commented that given the small number of replicate samples a statistical analysis was not possible. Germany felt that the study was not suitable for establishing intrinsic equivalence and therefore agreed with The Netherlands that specific recommendations should be drawn up indicating how such tests should be conducted.

III.5.3.4 Response by Syngenta – via The Netherlands, 29 November 2000

In its response to questions raised Syngenta provided an extensive overview of its numerous compositional analyses on Bt field maize and Bt sweet maize involving different inbreds and hybrids representing various stages in several independent breeding programs. According to Syngenta, to assess the potential for unintended pleiotropic effects, the 'transformation event Bt11' has to be considered as a whole, without distinction between sweet and field maize because corresponding maize plants have been used for comparison. The same goes for maize plants grown in greenhouses. In summary, the following components had been measured in the 'transformation event Bt11':

- Moisture, ash.
- Total protein or available protein or total nitrogen, and individually the amino acids Trp, Asp, Thr, Ser, Glu, Pro, Gly, Ala, Cys, Val, Met, Ile, Leu, Tyr, Phe, Lys, His, and Arg
- Fat, and individually palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, gadoleic acid, behenic acid.
- Total carbohydrates, or starch, sugars and fibres (either as total fibres, or as acidic dietary fibres and neutral dietary fibres).
- The anti-nutrients phytic acid and trypsin inhibitor.
- Xanthophyll, niacin, folic acid and the vitamins A (or rather pro-vitamin A), B1, B2, C, and E
- The minerals Ca, Cu, Fe, Mg, Mn, Na, P, and Zn
- The 5 secondary metabolites furfural, p-coumaric acid, ferulic acid, myo-inositol (phytic acid) and raffinose.

Syngenta noted that this list was much larger than the number of components recommended for analyses by the Nordic Council of Ministers in 1998, and included all the components recommended for maize. Syngenta was aware that the use of various hybrids, different experimental designs and different analytical methods makes a direct comparison of its numerous compositional analyses difficult. In the view of Syngenta, this was due to the fact that the requirements for compositional analyses evolved between 1995 and 2000. In addition, Syngenta provided the following justification for the choice of compounds for analysis apart from the obvious choices of proximates and amino acids. The selection was made either by Syngenta or by various competent authorities:

- The fatty acids chosen, and in particular the 5 fatty acids measured in various studies, represent the most common fatty acids found in maize, and account for 99% of the total lipids.
- The greatest proportion of carbohydrates in maize consists of starch, the rest being fibres and free sugars. Starch is preferably measured in kernels, whereas acid dietary fibres and neutral dietary fibres are preferably measured in whole plants.
- Phytic acid and trypsin inhibitor have been analysed for the reason that these compounds are known anti-nutrients, although they are present in low quantities in maize kernels.

- Maize is not an important dietary source of vitamins, and the content in vitamins varies significantly between different maize hybrids. Nevertheless, the major vitamins have been selected for measurements.
- The minerals Ca, Mg, P, K and K are required by the plants in significant quantities (macro-nutrients), and are directly incorporated into organic compounds synthesised by the plants. Some other minerals such as Fe and Zn are micro-nutrients, required only in small quantities.
- The secondary metabolites p-coumaric acid, ferulic acid, myo-inositol and raffinose have been selected for analyses because the concentration range of these compounds in maize grain is known. Furfural was selected because of its potential carcinogenicity.
- Sulphur was not measured directly in Bt11 maize. However, sulphur in maize kernels is largely present in organic form as a constituent of the amino acids methionine and cystine⁴¹. The levels of these two amino acids were not found to be significantly different between Bt11 maize hybrids and non-modified hybrids.
- The minerals Cl, I, Fl, Pb, Cd, Cr, Se, Co and Hg are normally not measured in maize kernels, although values for these compounds have been published. However, no values specific for sweet maize were available.
- Finally, Syngenta provided the raw data for the comparison of vitamins and mineral composition of grain from Bt11 maize and non-modified maize hybrids requested by Austria.

At the moment, it is not clear to SBC whether the applicant's response to questions on the safety evaluation by the Dutch authorities provided by the applicant satisfies the other member states, nor is it clear how this impacts regulatory decision-making on the food use of Bt11 sweet maize by the European Commission and the national authorities of EU member states.

III.5.4 EC Scientific Committee on Food, 7 September 2000

Based on safety assessments carried out by the UK ACNFP on MON810 maize (14.02.1997), T25 maize (14.02.1997), and Bt11 maize (14.02.1997) conducted prior to the introduction of Regulation 258/97, products thereof were notified as the companies claimed that they were substantially equivalent to existing products.

In 1998 SCP subsequently reviewed the safety of MON810 maize (10.02.1998), T25 maize (14.02.1998) and Bt11 maize (14.02.1998). On 4 August 2000 the Italian authorities informed the Commission that they considered that these products authorised under the notification procedure were not substantially equivalent within the meaning of Regulation (EC) 258/97 and should therefore be submitted to a full safety evaluation. In addition, the Italian authorities invoked the so-called 'safeguard clause' (article 12) and issued a decree suspending the trade and use of products derived from maize lines MON810, T25 and Bt11. Two reports were made available, of which the second one identifies some shortcomings in the original application, e.g. the lack of PCR data used currently to detect DNA from the transgenic plant in the product. Although such data would be required in any current application to support the establishment of substantial equivalence of the product to its conventional counterpart, the absence of such data is not evidence of a risk to health. The Committee was therefore of the opinion that the information from the Italian authority did not provide detailed scientific grounds for considering that the use of the novel foods in question endangers human health.

⁴¹ See footnote 36.

III.5.5 EC Scientific Committee on Plants, 30 November 2000

COMPONENT	DATA/SOURCE	REMARKS
Moisture, nitrogen, ash, starch, cellulose, xanthophyll, fatty acids, amino acids	Kernels from glasshouse cultivation in Europe	No significant differences
Protein, oil, starch, fibre	Kernels 4 Bt11 hybrids six field locations US	No significant differences apart from lower protein content of two BT11 hybrids related to backcrossing
Fatty acids, amino acids	Kernels 2 Bt11 hybrids six field locations US	Minor differences for palmitic acid, stearic acid, cystine and arginine
Cu, Mg, Mn, Zn	Kernels 2 Bt11 hybrids three field locations US	No significant differences
Folic acid, vitamin B1, vitamin B2	Kernels 2 Bt11 hybrids three field locations US	No significant differences
Carbohydrate, protein, fat, fibre, fatty acids, amino acids, trypsin inhibitor, and phytic acid	Kernels 3 Bt11 hybrids two field locations	No significant differences
Nitrogen, carbohydrate, lignin	Exp, silage of Bt11 US	Substantial equivalence

SCP did not explicitly determine the GM plant (and products thereof) as substantially equivalent to its conventional counterpart (except for the modified trait).

III.6 PROCESSED PRODUCTS OF TRANSFORMATION EVENT GA21

Plant: Maize
Main trait of interest: Glyphosate-tolerance
Applicant: Monsanto
Directive 90/220 nr.: C/ES/98/01 (uses as any other maize, received by the Commission on 20.05.1999, pending)
C/GB/97/M3/2 (import and processing, received by the Commission on 28.10.1999, pending)
Regulation 258/97: Request for authorisation pending;
Initial assessment by The Netherlands on 21.12.1999

Available documentation and information

1. Request for authorisation of products derived of GA21 submitted in The Netherlands. Initial assessment completed 21 December 1999; questions from member states.
2. Scientific Committee on Plants, 22 September 2000.

III.6.1 VNV, The Netherlands, 21 December 1999

On 24 July 1998 the applicant requested the Netherlands to *authorise* GA21 maize hybrids for food use. Also an opinion was requested on the substantial equivalence of oil and starch hydrolysates derived of GA21 maize. On 24 February 1999 the Committee Safety Evaluation of Novel Foods (Dutch acronym VNV) of the Health Council asked the applicant to provide additional data on the levels of five secondary plant metabolites, i.e. furfural, p-coumaric acid, ferulin acid, raffinose and myo-inositol. On 25 March 1999 the applicant argued that secondary plant metabolites such as solanin in potato, glucosinolates in oilseed rape and gossypol in cotton are nutritionally significant toxicants but as maize has a history of safe use, it would not be relevant to investigate the levels of the secondary plant metabolites requested by The Netherlands. The applicant also noted that although the presence of the EPSPS-enzyme may in theory disrupt the synthesis of aromatic amino acids like tyrosin, tryptophan and phenylalanin. But the levels of these aromatic amino acids were comparable between

GA21 maize hybrids and their controls. On 18 June 1999 the committee reiterated its request to forward data on the five secondary plant metabolites, which were subsequently provided by the applicant on 26 October 1999. Finally, in its advice on 21 December 1999, the committee indicated that the applicant clearly followed the flowcharts annexed to Regulation 258/97 and that the molecular-biological, nutritional and toxicological data provided are adequate for an evaluation by the committee.

III.6.1.2 Nutritional information

The applicant conducted nutritional analysis of maize kernels regarding protein, oil, carbohydrates, fibre, dry residue, moisture, fatty acids, amino acids, Ca and P. Other parts of the maize plants have also been analysed. For these analyses samples had been drawn from GA21 maize hybrids, their controls and other local maize varieties grown at five different locations in the US in 1996 and in 1997 at seven locations in the US and four locations in Europe. The composition of the GA21 maize hybrids appeared neither to differ from that of their conventional controls, nor from what literature⁴² reports. To determine the average values and the spread of a food component, data of samples from five locations had been pooled. From a nutritional perspective there was no difference between conventional maize and GA21 maize. The committee agreed with this conclusion.

III.6.1.3 Toxicological information

The *degree* of substantial equivalence of two GA21 maize hybrids and the conventional parent line has been underpinned by a comparison of the composition from a nutritional perspective and by a further analysis of five secondary plant metabolites, e.g. furfural, raffinose, phytic acid, p-coumarin acid and ferulin acid. The content of furfural was below the detection limit, whereas the contents of the other substances in the GA21 maize hybrids did not differ from those in conventional maize, apart from that of p-coumarin acid, which was statistically significantly higher in one of the GA21 maize hybrids. The applicant argued that this difference is small, fell within the range of values for other commercial maize varieties and was unlikely to be biologically significant. The committee agreed with this.

III.6.1.4 Conclusions and recommendations

According to the Dutch VNV, the modified maize hybrid differed from a conventional hybrid regarding the mEPSPS gene and the expression product of the modified gene. There were no indications that the genetic modification in the plant leads to adverse pleiotropic effects. The modified EPSPS protein had not been found toxic or allergenic at occurring concentrations. Variations of other maize components investigated fell within the range of values reported in literature and did not have consequence from a health perspective. The committee viewed this information complete and correct as far as it is relevant for a safety evaluation. The data submitted had been correctly interpreted and evaluated according to the present state of science. In the view of the committee, consumption of GA21 maize is as safe for humans as that of non-genetically modified maize and maize products. The committee also noted that applicants would be helped by concrete guidance concerning the number of samples, locations and years, which are needed for the quantitative analyses. The committee itself would elaborate on this recommendation and forward a proposal to international deliberations within the framework of implementing EU Regulation 258/97.

⁴² References: 1) Jugenheimer, R.w., Corn improvement, seed production, and uses, New York: John Wiley & Sons, Inc, 1976; 2) Us Department of Agriculture, USDA Handbook no 8, via Telnet, Mar 3, 1993; 3) Watson, S.A., Corn, amazing maize, general properties. In: Wolff L.A. (red.) CRC Handbook of processing and utilisation in agriculture, Vol II, part I, CRC Press, 1982: 3-29; and 4) Watson, S.A., Structure and composition. In: Watson, S.A. and Ramstad, R.E. (Eds.), Corn chemistry and technology, St Paul, Minnesota: Am Soc Cereal Chem, 1987: 3-29.

III.6.1.5 Questions raised by other member states

Denmark questioned whether the information provided on the nutritional values was sufficient to determine the substantial equivalence of GA21 maize. Greece thought the phenotypic analysis of the genetic stability convincing but saw one unexplained band on the Southern Blot. Also Ireland had small remarks on the genetic stability and noted that the studies on compositional analyses were limited regarding the characteristics measured and the number of locations and seasons. Italy felt that information on the levels of vitamins, minerals and protease inhibitor was lacking, whereas Austria wished in particular to receive the original data of the compositional analyses, in particular regarding tocopherols and phytic acid. Portugal wanted more details about the statistical analyses of the levels of secondary metabolites and data per plant and location, so as to filter extreme values. Sweden felt that the applicant had not sufficiently documented whether no unintended effect had occurred due to the genetic modification and missed analyses of the levels of Se, zeaxanthin and trypsin inhibitor.

At the moment, it is not clear to SBC whether the applicant has responded to questions on the safety evaluation by the Dutch authorities by the other member states, nor is it clear how this impacts regulatory decision-making on the food use of GA21 maize by the European Commission and the national authorities of EU member states.

III.6.2 EC Scientific Committee on Plants, 22 September 2000

Maize line GA21 and control lines, which are negative progeny of the same crossing event and do not contain mEPSPS gene, had been grown at five different locations in 1996. Grain samples were obtained from 9 to 16 ears and forage samples were composed of 2 to 4 plants at the soft dough stage without roots. In 1997 grain and forage sample from seven US locations and four European locations were assayed. In 1996 plants were not treated with glyphosate, whereas in 1997 the plants were treated with the herbicide.

COMPONENT	DATA/SOURCE	REMARKS
Ash, calcium, carbohydrates, acid detergent fibre ADF, neutral detergent fibre NDF, moisture Phosphorous, protein, fat, fatty acids, amino acids, trypsin inhibitors, phytic acid, and vitamin E	Grains and forage from GA21 maize lines on 11 locations in 1996 and 1997	No statistical differences found. Using animal feeding studies, the data assess, very clearly demonstrated the nutritional equivalence of grain to isogenic material, on the basis of growth performance and body composition of broilers receiving GM maize compared to isogenic grain for 40 days.

To the opinion of SCP, these data satisfactorily demonstrated the substantial equivalence of the GA21 maize to its conventional counterparts.

III.7 PROCESSED PRODUCTS OF TRANSFORMATION EVENT T25

Plant: Maize
Main trait of interest: Glufosinate-tolerance
Applicant: AgrEvo
Directive 90/220 nr.: C/F/95/12/07 (use as any other maize, approved 22.04.1998)
Regulation 258/97: Notification transmitted to Member States on 06.02.1998

Available documentation and information

1. Products derived of T25 notified by United Kingdom on 12 January 1998.
2. Scientific Committee on Plants, 10 February 1998.
3. Opinion of the Scientific Committee on Food concerning a submission from the Italian authorities raising concerns for the safety of certain products approved under the notification procedure of Regulation (EC) 258/97, 7 September 2000.

III.7.1 ACNFP, United Kingdom, 12 January 1998

In January 1998, on the basis of data on the contents of fatty acids, protein, amino acids, crude fibre, ash, phytate and moisture, the UK authority concluded that there had been no unintentional changes in the composition of T25 maize, although for some parameters variation between the GM and non-GM lines has been observed. However, as there was little experience in predicting the effect of genetic drift on the metabolism of any line of plants, whether GM or non-GM, the UK authority asked that the seed composition and the fatty acid and amino acids profiles of T25 maize lines should be monitored over time.

III.7.2 Scientific Committee on Plants, 10 February 1998

COMPONENTS	DATA/SOURCE	REMARKS
Fatty acid, protein, amino acids, crude fibre, ash, phytic acid, and moisture	Grain and silage maize from field trials in US and Europe	The integration of the PAT gene into the maize genome and the expression of PAT protein do not seem to cause any negative pleiotropic effects on the plant characteristics to its safety for humans and animal consumption, i.e., the nutritional composition and the content of the natural anti-nutritional factors are within the normal range.

The SCP concluded that the GM maize plant was substantially equivalent to its non-GM counterpart except for the introduced trait.

III.7.3 EC Scientific Committee on Food, 7 September 2000

Based on safety assessments carried out by the UK ACNFP on MON810 maize (14.02.1997), T25 maize (14.02.1997), and Bt11 maize (14.02.1997) conducted prior to the introduction of Regulation 258/97, products thereof were notified as the companies claimed that they were substantially equivalent to existing products.

In 1998 SCP subsequently reviewed the safety of MON810 maize (10.02.1998), T25 maize (14.02.1998) and Bt11 maize (14.02.1998). On 4 August 2000 the Italian authorities informed the Commission that they considered that these products authorised under the notification procedure were not substantially equivalent within the meaning of Regulation (EC) 258/97 and should therefore be submitted to a full safety evaluation. In addition, the Italian authorities

invoked the so-called 'safeguard clause' (article 12) and issued a decree suspending the trade and use of products derived from maize lines MON810, T25 and Bt11. Two reports were made available, of which the second one identifies some shortcomings in the original application, e.g. the lack of PCR data used currently to detect DNA from the transgenic plant in the product. Although such data would be required in any current application to support the establishment of substantial equivalence of the product to its conventional counterpart, the absence of such data is not evidence of a risk to health. The Committee was therefore of the opinion that the information provided by the Italian authorities did not provide detailed scientific grounds for considering that the use of the novel foods in question endangers human health.

III.8 PROCESSED PRODUCTS OF TRANSFORMATION EVENT MON810

Plant: Maize
Main trait of interest: Insect-resistance
Applicant: Monsanto
Directive 90/220 nr.: C/F/95/12/02 (use as any other maize, approved 22.04.1998)
Regulation 258/97: Notification transmitted to Member States on 06.02.1998

Available documentation and information

1. Initial assessment by ACNFP, United Kingdom, 10 December 1997
2. Opinion of Scientific Committee on Plants issued on 10 February 1998

III.8.1 ACNFP, United Kingdom, 10 December 1997

The applicant provided data from the compositional analyses regarding fatty acid, protein, amino acid, crude fibre, ash and moisture content of grain and forage GM and non-GM maize grown in agronomic fields tests in Europe and in the USA. According to ACNFP, there had been no unintentional changes in the composition of the grain from the GM plants nor in the plants themselves as a result of the genetic modification. It concluded that the composition of the GM maize does not differ from that of conventionally bred maize.

However, there was little experience in predicting the effect of genetic drift on the metabolism of any line of plants, whether genetically modified or conventionally bred. Therefore ACNFP asked the applicant to monitor the seed composition, including amino acid and fatty acid profiles of the oil from MON810 over time.

III.8.2 EC Scientific Committee on Plants, 10 February 1998

COMPONENTS	DATA/SOURCE	REMARKS
Fatty acids, protein, amino acids, crude fibre, ash, phytic acid, and moisture	Grain and silage from field trials in the US and Europe	No significant nutritional differences could be detected.

The Committee determined the GM maize line substantially equivalent to non-GM maize except for the transferred traits.

III.8.3 EC Scientific Committee on Food, 7 September 2000

Based on safety assessments carried out by the UK ACNFP on MON810 maize (14.02.1997), T25 maize (14.02.1997), and Bt11 maize (14.02.1997) conducted prior to the introduction of

Regulation 258/97, products thereof were notified as the companies claimed that they were substantially equivalent to existing products.

In 1998 SCP subsequently reviewed the safety of MON810 maize (10.02.1998), T25 maize (14.02.1998) and Bt11 maize (14.02.1998). On 4 August 2000 the Italian authorities informed the Commission that they considered that these products authorised under the notification procedure were not substantially equivalent within the meaning of Regulation (EC) 258/97 and should therefore be submitted to a full safety evaluation. In addition, the Italian authorities invoked the so-called 'safeguard clause' (article 12) and issued a decree suspending the trade and use of products derived from maize lines MON810, T25 and Bt11. Two reports were made available, of which the second one identifies some shortcomings in the original application, e.g. the lack of PCR data used currently to detect DNA from the transgenic plant in the product. Although such data would be required in any current application to support the establishment of substantial equivalence of the product to its conventional counterpart, the absence of such data is not evidence of a risk to health. The Committee was therefore of the opinion that the information provided by the Italian authorities did not provide detailed scientific grounds for considering that the use of the novel foods in question endangers human health.

III.9 PROCESSED PRODUCTS OF CONVENTIONAL CROSSES T25 AND MON810

Plant: Conventional crosses of GM maize lines T25 and MON810
Main trait of interests: Glufosinate-tolerance and insect-resistance
Applicant: Pioneer Hi-Bred
Directive 90/220 nr.: C/NL/98/08 (uses as any other maize, received by the Commission on 24.04.1999, pending)
Regulation 258/97: Request for authorisation pending; initial assessment by The Netherlands pending.

Available documentation and information

1. The 1999 ACNFP annual report, United Kingdom.
2. Request for authorisation submitted in The Netherlands on 28 April 2000.
3. Scientific Committee on Plants issued on 6 June 2000.

III.9.1 Crossing of two GM plants, ACNFP, 1999

In the ACNFP 1999 annual report, this Committee indicated that it is routinely consulted by ACRE, the UK advisory body for applications under Directive 90/220, particular in relation to molecular biology aspects and their implications, if any, for the food chain arising from the commercial release of a GM variety. In 1999 ACNFP received information on an application for a maize hybrid derived from the crossing of two approved GM lines under this directive. ACNFP recognised this application as the first⁴³ of many applications from a product derived from crossing of two GM varieties. In this case, according to ACNFP, the applicant had heavily relied on the fact that the two GM parents of the proposed hybrid had already been approved, and so very little information on the new hybrid was provided. However, ACNFP challenged the assumption that the hybrid would exhibit the same properties as the GM parent lines. Therefore any of such crosses would, at this stage, need to be assessed on a

⁴³ According to "Facts on GMOs in the EU" (European Commission, MEMO/00/43, Brussels, 13 July 2000), these approved GM parent lines may concern T25 GM maize transformation event (C/F/95/12/07), respectively MON810 GM maize transformation event (C/F/95/12-02), both approved for commercial release as of 22 April 1998. The application for commercial release of the hybrid T25xMON810 under Directive 90/220 (C/NL/98/08) has been received (via The Netherlands) by the European Commission on 29 April 1999. This application is pending.

case-by-case basis in the same way as any other product from a GM variety. In particular, the possibility of unintended effects arising from insertion events brought about by the crossing could not be ruled out. ACNFP agreed that approval could not currently be based solely on the properties of the parent line. Further, in all cases a precautionary approach should be adopted. ACNFP noted that in other countries, such as the USA, clearance of a GM line also covered further crosses with approved GM lines.

III.9.2 VNV, The Netherlands, 28 April 2000

A request for *authorisation* has been submitted in The Netherlands on 28 April 2000. The initial assessment is currently being prepared by Committee Safety Evaluation of Novel Foods (Dutch acronym VNV) of the Health Council.

III.9.3 EC Scientific Committee on Plants, 6 June 2000

COMPONENT	DATA/SOURCE	REMARKS
Protein, fibre, fat, moisture, ash, fatty acids, and amino acids	US field trial in 1997	No significant differences between T25, MON810, T25xMON810 and control line (hybrid 3394) apart from small differences in fatty acid composition
Protein, fibre, fat, moisture, ash, fatty acids, and amino acids, Ca, K, Mg, Mn, Na, P, folic acid, thiamine, riboflavin, vitamin E, phytic acid and trypsin inhibitors	Field trials on 4 locations in Italy in 1998	No significant differences between T25xMON810 and control line (hybrid X1106RT) apart from significant but small differences for grain ash, stearic acid, linolenic acid, arginine, and riboflavin; levels are however within ranges published for maize.

The SCP concluded that the compositional data (...) demonstrate the substantial equivalence of hybrid T25xMON810 to its parent lines T25 and MON810 and to the non-transgenic conventional maize except for the transferred traits.

III.10 PROCESSED PRODUCTS OF TRANSFORMATION EVENT TGT7F

Plant: Tomato
Main trait of interest: Down-regulation of polygalacturonase
Applicant: Zeneca
Directive 90/220 nr.: C/ES/96/01 (uses as any other processing tomato, received by the Commission on 24.11.1997, pending)
Regulation 258/97: Authorised by the UK in 1996;
 Request for authorisation by EU pending.

Available documentation and information

1. ACNFP annual report, Annex II, United Kingdom, 1997.
2. Assessment ACNFP by United Kingdom in 1998.
3. Scientific Committee on Plants issued on 23 June 1998.
4. Opinion of Scientific Committee on Foods issued on 23 September 1999.

5. Exemplary case study by the Institute of Toxicology of Danish National Food Agency in OECD Safety Evaluation of Foods derived by Modern Biotechnology, 1993.⁴⁴

III.10.1 ACNFP, United Kingdom, 1997

According to the Advisory Committee on Novel Foods and Processes (ACNFP) annual report of 1997, the original submission was received in August 1994. In February 1995 ACNFP published its report detailing clearance of the food use of paste from two GM hybrid lines derived from the GM inbred line TGT7F. In August 1995 the applicant requested to extend the scope of the original clearance to tomato paste and processed tomato products containing the paste (ketchup, pizza sauces) from three additional GM lines. The applicant also sought a much broader food safety clearance of tomato paste and its products from any tomato hybrid line derived from TGT7F, and any tomato lines genetically modified with construct pJR16S. In February 1996 ACNFP advised to extend the food safety clearance of paste and products containing the paste from any tomato hybrid line derived of TGT7F but not to any inbred or hybrid lines containing the construct pJR16S obtained from a different transformation event.

Comparative nutritional and toxicological analyses had been carried on the diced and chopped GM tomatoes packed in tomato juice produced during the 1995 Italian trials. These had been grown under the same conditions at the same time and harvested and processed in the same way as the non-GM controls. Two commercially available products were also analysed. ACNFP was satisfied that the nutritional values of the GM tomato products fell within the range of the controls. Data on 4 known, naturally occurring toxins (alpha-tomatine, solanine, chaconine and nicotine) indicated that their levels in both the GM and non-GM control samples were below the level of detection. ACNFP was therefore satisfied that the nutritional and toxicological composition of the peeled products from the GM tomatoes was comparable to products from conventionally bred tomatoes consumed as part of the UK diet.

III.10.2 ACNFP, United Kingdom, 1998

In March 1998 the applicant submitted an application for a safety evaluation to the ACNFP. Prior to the introduction of EU Regulation 258/97 ACNFP had evaluated the GM tomatoes and their products under UK's voluntary system. Tomato paste produced from the GM tomatoes had been on sale in the UK since February 1996. As not all the processed products produced from the GM tomatoes cleared under the UK's voluntary system had been marketed, it was necessary to carry out a full assessment as required by Regulation 258/97.

III.10.2.1 Nutritional information

Comparative nutritional analysis on inbred and hybrid varieties of the GM line of both the fresh fruit and the processed products and in each case the appropriate non-GM controls and commercially available products were also compared. A comparison of the result with published ranges was also presented. The following nutritional components were analysed:

- Moisture, ash, fat total carbohydrate, total dietary fibre, soluble fibre, insoluble fibre, total sugars, protein, cellulose, hemicellulose and energy value.
- Na, K, Ca, Mg, P and Fe.
- Vitamins A, E, B1, B2, niacin, B6, folic acid, and C.
- Malic acid, citric acid and lycopene.

The results showed that the genetic modification did not change the nutritional profile of the processed products.

⁴⁴ See footnote 4.

III.10.2.2 Toxicological information

The tomato is known to have the potential to accumulate naturally occurring toxins known as glycoalkaloids. The principal naturally occurring toxin in tomato is alpha-tomatine, but the principal alkaloids of potato (solanine and chaconine) have also been found in tomatoes in low amounts. Tests conducted showed that the levels of glycoalkaloid toxins in the GM fresh fruit were below their respective levels of detection. The levels in the processed products were also below the level of detection except for alpha-tomatine, which had a level of 58 mg/kg in the GM inbred line, although this was below that of the non-GM control, which had levels of 74 mg/kg.

Other naturally occurring toxins such as tyramine, serotonin, histamine, nicotine and lectins were also analysed and their levels in GM tomato paste were comparable with the commercial controls and were often below the level of detection. This evidence demonstrated that the introduced genes had not had any effect on the levels of naturally occurring toxins. Also analysis for toxic metals like As, Cd, Pb and Hg have been conducted, as metal contamination of plants may arise naturally from the nature of the soil in which they grow or other agronomic practices. The results showed that the level of toxic metal contamination of the GM fruit and products fell within the range of values obtained in the controls and commercially available tomato products. Further there were no reports found that the nptII gene and its protein are inherently toxic. The protein is also heat labile and degrades rapidly in the human gut. Additional information presented by the applicant demonstrated that no detectable amounts of the nptII gene encoded protein were present in processed products from the GM tomato.

The compositional (nutritional and toxicological) data satisfied ACNFP that no differences existed between the GM tomato fruit or the processed products produced from them and their non-GM counterparts. This was further evidence that no secondary effects had occurred as a result of the genetic modification.

III.10.3 EC Scientific Committee on Plants, 23 June 1998

COMPONENT	DATA/SOURCE	REMARKS
Soluble sugars, structural carbohydrates (dietary fibre), moisture, ash, fat, protein, oil, Na, K, Ca, Mg, P, Fe, vitamins (A, E, B1, B2, niacin, B6, folic acid, C), lectins, alpha-tomatine, solanine, chaconine, tyramine, nicotine and serotonin	Fresh fruit and tomato paste samples compared to the range of values produced by non-modified, commercial tomato varieties	Ranges fall within ranges expected of commercial tomato fruit and fruit purees

The SCP determined harvested GM fruit as substantially equivalent to the fruit of other commercial processing tomatoes.

III.10.4 EC Scientific Committee on Food, 23 September 1999

The establishment of substantial equivalence is one of the key issues for the scientific assessment of the degree of safety of GM food, although not being a safety assessment itself. No toxicological studies had been performed by the applicant. The application did, however, correspond to a GM food for which substantial equivalence to its conventional counterpart could be demonstrated with regard to the genetic, agronomic, nutritional and

compositional characteristics of the GMO with the differences being those triggered by the insertion of specific DNA.

The SCF concluded that TGT7F is substantial equivalence to the unmodified and conventional counterparts had been established for the GM tomato raw fruit except for the specified traits, as well as for processed products.

III.10.5 Theoretical case study, OECD 1993

In one of the first OECD documents, in which the concept of substantial equivalence was introduced as a regulatory tool in the food safety assessment of GM foods, several case studies were provided as an illustration of how this concept could be applied. In the case study on GM tomato, the Danish representatives indicated that conventional tomato varieties were frequently tested for cultivar examination in their country. Tomato was also included in the Danish Food Monitoring System, as tomato was regarded as an important food for the intake of certain nutrients like vitamin C, folacin, vitamin B1 and vitamin B6. At that time, no analysis of levels of natural toxins were included, partly due the lack of approved analytical methods and partly because no real concern had been expressed. However, the need for such studies should be considered in order to determine a 'baseline' level of natural tomato toxins. Following compounds were listed as toxic constituents in tomato: alpha-tomatine, tomatidine aglycone of tomatine, saponines, coumarins, lectins, serotonin, oxalate, protease-inhibitor and histamine.

Furthermore, with regard to the example of GM tomato with prolonged fruit ripening through using an antisense RNA technique (as in the GM tomato developed by Zeneca), it was expected that any secondary changes would not occur because such a GM tomato would develop normally but more slowly.