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Federal Environment Agency – Austria

EVALUATING SUBSTANTIAL EQUIVALENCE

A step towards improving the risk/safety evaluation of GMOs

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INTRODUCTION

The concept of Substantial Equivalence was introduced into the discussion of safety evaluation of food from genetically modified organisms for the first time in 1993.[1] Subsequently, the concept was agreed in many countries as a basis for safety evaluation of novel food.[2] Substantial Equivalence in this regard means that a genetically modified plant or food derived therefrom is equivalent to their conventional counterparts. Substantial Equivalence is determined by comparing plant compounds as well as agronomic and morphologic properties. In case of significant differences further testing will be decided on a case-by-case basis. Thus, the concept of Substantial Equivalence represents an important part of safety evaluation of food produced from genetically modified organisms.

However, Substantial Equivalence is also a controversial issue because of more fundamental considerations and also because of the way the concept is applied.[3] Some major criticism is briefly described in the following:

The allergological and toxicological safety of genetically modified plants and food derived therefrom cannot be reliably concluded solely from chemical composition analysis depending itself on very limited number of plant compounds. This approach ignores the possible appearance or increased accumulation of new allergens or toxins as a results of non-intended secondary effects. The use of new methods such as mRNA-fingerprinting, proteomics, DNA-array, chemical fingerprinting[4] have to be considered, especially for the next generation of genetically modified plants, as their ingredients might be quantitatively or qualitatively engineered in a more complex way compared to present examples.

Some cases of practicing Substantial Equivalence are controversial and did possibly lead to safety declarations which are not fully verifiable or which at least resulted in a short cut in safety evaluation, which seems not to be justifiable.[5] As the criteria used for Substantial Equivalence are of considerable importance for the decision of whether or not and what particular kind of further testing will be required in the course of safety evaluation, there is an increasing demand that the criteria for Substantial Equivalence should be reassessed and also possibly complemented.

Besides such conceptual considerations and criticism of the practice of Substantial Equivalence, the results of the first systematic and comparative investigations of the use of Substantial Equivalence in the practice of safety evaluations were recently presented. These results clearly show that there are actual shortcomings and there is in fact a demand for further shaping or re-shaping this concept.[6]

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[1] OECD 1993: Safety Evaluation of Foods derived by Modern Biotechnology. Concepts and principles. OECD, Paris.

[2] WHO: 1995: Application of the Principle of Substantial Equivalence to the Safety Evaluation of Foods and Food Components from Plants derived by Modern Biotechnology, Report of a WHO Workshop. WHO, Geneva; Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. Official Journal L 043 , 14/02/1997 p. 0001–0006.

[3] E.g. Millstone, E.; Brunner, E.; Mayer, S. 1999: Beyond “substantial equivalence”. Nature 401, p.525-526; OECD 2000: Report of the Task Force for the Safety of Novel Foods and Feeds to the G 8, C(2000)86/ADD1.

[4] Kuiper, H.A.; Noteborn, H.P.J.M.; Peijnenburg, A.A.C.M. 1999: Commentary: Adequacy of methods for testing the safety of genetically modified foods. The Lancet 354, S.1315-1316.

[5] Greenpeace 1996: Roundup Ready Soybean. Schwerwiegende Mängel der Monsanto-Risikoeinschätzung zu den Gefahren durch die Freisetzung genmanipulierter Sojabohnen mit Glyphosat-Resistenz, die der EU für die Zulassung vorgelegt wurden. Greenpeace; sowie der Fall der transgenen Lektinkartoffeln.

[6] E.g. Nordic Report 1998 according to FAO/WHO 2000: Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology. Topic 2: Application of Substantial Equivalence. Data Collection and Analysis; Novak, W.K. and Haslberger, A.G. 2000: Substantial Equivalence of Antinutrients and Inherent Plant Toxins in Genetically Modified Novel Foods. Food and Chemical Toxicology 38, p.473-483; Fuchs, R. Assessing the Substantial Equivalence of Monsanto’s Biotechnology Products. In: OECD 1998: Report of the OECD Workshop on the toxicological and nutritional testing of novel Foods. OECD SG/ICGB(98)1, p.24-25.

The situation described above served as a starting point and built the context of the workshop "Evaluating Substantial Equivalence" organised by the Austrian Federal Environment Agency (UBA), Vienna, and the Inter-University Research Center for Technology, Work and Culture (IFF/IFZ), Graz, in Vienna from October, 19th to 20th 2001. Major goals of this workshop were on the one hand to evaluate experience gained so far in applying the concept and on the other hand to find out about the problems and obstacles in operationalizing the concept. The use of the concept in connection with toxicological and allergological safety evaluation was of particular interest in the course of the workshop.

The workshop was embedded in two UBA-IFF/IFZ projects commissioned by the Federal Ministry of Economy and Labour and the Federal Ministry of Social Security and Generations entitled "Standardization of Toxicological and Allergological Safety Evaluation of GMO Products". In the course of these projects the application dossiers containing the safety evaluations for genetically modified plants according to EU Directive 90/220/EEC (project 1) and according the EU Novel Food Regulation (project 2) were investigated. These investigations focus on the performed tests and presentation of results, consideration of exposure (which may vary according to the intended use), and the line of reasoning given. On the basis of these results and as a second step conclusions will be drawn and recommendations made in order to improve, concretise and standardize safety evaluation.

More than 50 experts from university, industry, competent authorities, international organisations, and NGOs representing environmental and consumer interests from Belgium, Canada, Germany, France, Italy, the Netherlands, Norway, Slovenia, Sweden, United Kingdom and Austria took part in this workshop.

On the first day of the workshop different interpretation routes of the concept were elucidated, conceptual criticism was discussed, and the perspective of industry was presented. The practice of Substantial Equivalence was evaluated and the importance of secondary effects and of the traditional counterpart were discussed on the second day.

The proceedings in hand, issued by the organisers, includes the papers presented at the workshop as far as they were completed in writing by the speakers.

From the perspective of the editors the following conclusion can be drawn: Substantial Equivalence – as concept of relative safety – is still a highly controversial issue. However, this concept could be more easily implemented compared with concepts aiming at an absolutely defined level of safety. Criticism as mentioned above was largely endorsed in the workshop and aims on the one hand on the normative premises of the concept, e.g. the acceptability of the safety of traditional counterparts and on the reliability of conclusions drawn, e.g. the conclusion of toxicological and immunological safety on the basis of partial compositional analysis. On the other hand criticism on the practice of Substantial Equivalence was considerably based on the evaluation studies investigating application dossiers according to the EU Novel Food Regulation or to the EU Directive 90/220/EEC. According to these studies the use of the concept differs a lot between the applicants, the line of reasoning and the interpretation of testing results cannot be verified in each case and has to be questioned.

Furthermore, a learning process in the competent authorities and committees can be observed, which became noticeable as the basis and criteria of their decisions changes in time. This process also influenced the interpretation routes for Substantial Equivalence in the EU.

Despite these criticism and problems in practice it seems that the majority of experts still sticks to this concept – also because there is no feasible alternative approach in sight. Whether in some areas e.g. allergology, the approach of reasoning could be complemented or maybe replaced by simple standard testing remains to be controversial.

Finally, the experts are very aware of the problems and the need for action. The challenges to be met in order to base the concept more on accepted scientific principles, lie within the non-intended secondary effects and in establishing the foundations of and the specifications for the comparative analysis of genetically modified plants with their traditional counterparts.

In order to detect potential secondary effects in genetically modified organisms by means of e.g. profiling techniques, intense research work remains to be done, as the data generated by such methods has to be understood as well. Moreover, these techniques have to be further developed in order to achieve standardised and validated testing methods. Although considerable efforts have been made in the EU to approach this goal, the routine use of such techniques is still not in sight. Regarding the conventional counterpart, there is a substantial lack of knowledge on environmentally induced gene expression and plant metabolism. Furthermore, data on plant compounds and natural ranges thereof should be collected and made available in databases.

Without any doubt the further improvement, harmonisation and concretising of safety evaluation of GMO products in general and of the concept of Substantial Equivalence in particular, is of great importance. Guaranteeing a high level of safety is a key prerequisite in the context of further development, commercialisation and diffusion of genetic engineering and thereby a key factor in order take advantage of the innovational and economic potential.

Thus, approaches from OECD and EuropaBio to establish consensus documents, guiding the safety evaluation of each particular crop, e.g. recommending key compounds and natural ranges thereof are highly welcome. The development of profiling techniques, as carried out for instance in the context of the ENTRANSFOOD network of the EU are just as important. Furthermore, it is important to learn from the practice of the application of the concept of Substantial Equivalence as is the aim of the UBA-IFF/IFZ projects described above. The discussions and results of this workshop will significantly contribute to these projects which will be completed mid 2002 (project 1) and end 2002 (project 2).

EINLEITUNG

Das Konzept der „Substanziellen Äquivalenz“ wurde erstmals 1993 im Zusammenhang mit der Bewertung von Lebensmitteln aus gentechnisch veränderten Organismen in eine breitere Diskussion eingebracht[1] und in der Folge sowohl in zahlreichen Ländern als Grundlage für die Sicherheitsbewertung von derartigen Lebensmitteln und Lebensmittelzutaten etabliert.[2] Substanzielle Äquivalenz bedeutet in diesem Kontext die wesentliche Gleichwertigkeit einer transgenen Pflanze bzw. eines Lebensmittels oder einer Lebensmittelzutat, welche(s) aus transgenen Pflanzen hergestellt worden ist, mit der jeweiligen konventionellen Pflanze bzw. dem konventionellen Lebensmittel oder der Lebensmittelzutat. Die substanzielle Äquivalenz wird im wesentlichen durch einen chemisch-analytischen Vergleich sowie agronomische und morphologische Charakteristika bestimmt. Unterscheiden sich das Lebensmittel aus gentechnisch veränderten Pflanzen und das konventionelle Pendant, wird in Abhängigkeit vom Ausmaß des Unterschieds und auf einer case-by-case Basis über weitere erforderliche Untersuchungen entschieden. Somit stellt das Konzept ein Kernstück der Sicherheitsbewertung von Lebensmittelprodukten aus gentechnisch veränderten Organismen dar.

Dieses Konzept ist sowohl aus grundsätzlichen Übergelungen als auch durch die Handhabung in der Praxis heftig umstritten.[3] Folgende Kernpunkte der Kritik lassen sich identifizieren:

Aus der bloßen chemisch-analytischen Vergleichbarkeit *einiger ausgewählter Inhaltsstoffe* könne angesichts der Möglichkeit des erstmaligen oder verstärkten Auftretens von neuen Allergenen, Toxinen oder antinutritiven Substanzen (z. B. durch pleiotrope Effekte) nicht auf die allergologische und toxikologische Unbedenklichkeit der neuen Pflanze bzw. des neuen Lebensmittels *insgesamt* geschlossen werden. Speziell für die nächste Generation von Lebensmitteln aus gentechnisch veränderten Organismen, bei denen gezielte qualitative und/oder quantitative Veränderungen der Inhaltsstoffe vorgenommen werden, soll das Ausmaß und die Art der Untersuchungen zur Feststellung der Substanziellen Äquivalenz sowie der Einsatz von neuen Methoden wie z. B. mRNA-fingerprinting, proteomics, DNA-array, chemical fingerprinting, überdacht werden.[4]

Konkrete Fälle der Anwendung des Prinzips sind umstritten und haben möglicherweise zu *nicht nachvollziehbaren Unbedenklichkeitserklärungen* oder zumindest zum Verzicht auf weiterführende Untersuchungen im Rahmen der Sicherheitsbewertung geführt.[5] Es wird daher zunehmend gefordert, die Kriterien für Substanzielle Äquivalenz zu überdenken und gegebenenfalls zu ergänzen, da diese eine wesentliche Rolle für die Entscheidung spielen, *ob* und *welche weiteren* Untersuchungen erforderlich sind.

Neben diesen konzeptionellen Überlegungen und der Kritik an der Praxis der Sicherheitsbewertung liegen nun auch erste systematische und vergleichenden Untersuchungen der Anwendung des Prinzips im Rahmen der Sicherheitsbewertung von „Gentechnikprodukten“ vor.

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[1] OECD 1993: Safety Evaluation of Foods derived by Modern Biotechnology. Concepts and principles. OECD, Paris.

[2] WHO: 1995: Application of the Principle of Substantial Equivalence to the Safety Evaluation of Foods and Food Components from Plants derived by Modern Biotechnology, Report of a WHO Workshop. WHO, Geneva; Verordnung (EG) Nr. 258/97 des Europäischen Parlaments und des Rates vom 27. Januar 1997 über neuartige Lebensmittel und neuartige Lebensmittelzutaten.

[3] Stellvertretend: Millstone, E.; Brunner, E.; Mayer, S. 1999: Beyond “substantial equivalence”. Nature 401, S.525-526; OECD 2000: Report of the Task Force for the Safety of Novel Foods and Feeds to the G 8, C(2000)86/ADD1.

[4] Kuiper, H.A.; Noteborn, H.P.J.M.; Peijnenburg, A.A.C.M. 1999: Commentary: Adequacy of methods for testing the safety of genetically modified foods. The Lancet 354, S.1315-1316.

[5] Greenpeace 1996: Roundup Ready Soybean. Schwerwiegende Mängel der Monsanto-Risikoeinschätzung zu den Gefahren durch die Freisetzung genmanipulierter Sojabohnen mit Glyphosat-Resistenz, die der EU für die Zulassung vorgelegt wurden. Greenpeace; sowie der Fall der transgenen Lektinkartoffeln.

Auf Grundlage dieser Untersuchungen lassen sich Konkretisierungsbedarf und Schwachstellen in der Praxis der Handhabung des Konzeptes ausmachen.[6]

Diese Situation bildeten Ausgangspunkt und inhaltlichen Kontext für den vom Umweltbundesamt (UBA) Wien und dem Interuniversitären Forschungszentrum für Technik, Arbeit und Kultur (IFF/IFZ) Graz veranstalteten Workshops „Evaluating Substantial Equivalence", der vom 19. bis 20. Oktober 2001 in Wien stattfand. Ziel des Workshops war es, ein Resümee zu den bisherigen Erfahrungen mit der Anwendung dieses Konzepts zu ziehen und Problembereiche in der Operationalisierung zu identifizieren. Einen Schwerpunkt bildet dabei die Anwendung des Konzepts im Zusammenhang mit der toxikologischen und allergologischen Sicherheitsbewertung.

Der Workshop war eingebettet in zwei von UBA und IFF/IFZ im Auftrag des Bundesministeriums für Wirtschaft und Arbeit und des Bundesministeriums für Soziale Sicherheit und Generationen durchgeführten Projekte „Standardisierung der Vorgangsweise zur Abschätzung möglicher toxischer oder allergener Auswirkungen von GVO-Produkten". Im Rahmen dieser Projekte wird in einem ersten Schritt die toxikologische und allergologische Sicherheitsbewertung in den Antragsunterlagen für das Inverkehrbringen von gentechnisch veränderten Pflanzen nach der Richtlinie 90/220/EWG (Teil 1) und auf Lebensmittelprodukte aus gentechnisch veränderten Pflanzen nach der Novel Food Verordnung (Teil 2) untersucht. Im Zentrum der Untersuchung stehen dabei die durchgeführten bzw. dargestellten Tests, die Darstellung der Daten, die Berücksichtigung der – je nach Anwendungszweck – unterschiedlichen Exposition und die Argumentationsführung. Auf dieser Grundlage werden dann in einem zweiten Schritte Vorschläge zu einer Verbesserung, Konkretisierung und Standardisierung der Sicherheitsbewertung formuliert.

Insgesamt nahmen mehr als 50 ExpertInnen aus der universitären Wissenschaft, aus Industrie und Verwaltung, von internationalen Organisationen, sowie aus Umwelt- und Konsumentenschutzorganisationen aus Belgien, Deutschland, Frankreich, Kanada, Italien, Norwegen, Slowenien, Schweden, den Niederlanden und Großbritannien sowie aus Österreich am Workshop teil.

Am ersten Tag des Workshops wurden allgemeine Aspekte des Konzepts der Substanziellen Äquivalenz diskutiert, unterschiedliche Interpretationsformen sichtbar gemacht sowie konzeptionelle Kritik und die Perspektive der Industrie diskutiert. Am zweiten Tag standen die Präsentationen von Praxisevaluierungen, die Frage der Sekundäreffekte und des traditionellen Vergleichprodukts im Vordergrund.

Der vorliegende Band, herausgegeben von den Veranstaltern, gibt nun einen Überblick über die Beiträge im Rahmen dieses Workshops, soweit diese in schriftlicher Form von den ReferentInnen ausgearbeitet worden waren.

Zusammenfassend kann aus Sicht der Herausgeber folgendes festgehalten werden: Substanzielle Äquivalenz ist als Konzept einer relativen Sicherheit umstritten, aber – gegenüber einer Feststellung von absoluten Sicherheitsstandards – realistischer zu operationalisieren. Die eingangs erwähnte Kritik wurde im Rahmen des Workshops weitgehend bekräftigt und bezieht sich zum einen auf die normativen Grundlagen des Konzepts, wie z. B. die Akzeptabilität der Sicherheit von traditionellen Vergleichsorganismen bzw. -produkten sowie auf die Zulässigkeit von Schlussfolgerungen, wie z. B. der Schluss von sehr begrenzten chemischen Inhaltsanalysen auf toxische/immunologische Unbedenklichkeit insgesamt. Zum anderen er-

[6] Beispielhaft: Nordic Report 1998 zitiert nach FAO/WHO 2000: Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology. Topic 2: Application of Substantial Equivalence. Data Collection and Analysis; Novak, W.K. und Haslberger, A.G. 2000: Substantial Equivalence of Antinutrients and Inherent Plant Toxins in Genetically Modified Novel Foods. Food and Chemical Toxicology 38, S.473-483; Fuchs, R. Assessing the Substantial Equivalence of Monsanto's Biotechnology Products. In: OECD 1998: Report of the OECD Workshop on the toxicological and nutritional testing of novel Foods. OECD SG/ICGB(98)1, S.24-25.

hält die Kritik an der Praxis der Anwendung des Konzepts der Substanziellen Äquivalenz neue Impulse, etwa durch die Evaluierungen von Antragsunterlagen nach der Novel Food Verordnung und der Richtlinie 90/220/EWG. Diese Praxis zeichnet sich dadurch aus, dass die Antragsteller in sehr unterschiedlicher Weise mit dem Konzept umgehen, und dass manche Argumentationsweisen und Untersuchungen nicht nachvollziehbar bzw. in Frage zu stellen sind.

Darüber hinaus ist ein „Lernprozess" der begutachtenden Behörden und Komitees festzustellen, der sich in der Änderung von Entscheidungsgrundlagen und Beurteilungskriterien niederschlägt. Durch letzteren Prozess hat sich in der EU auch die Interpretationsweise des Konzepts verändert.

Trotz aller Kritik und Umsetzungsprobleme solle jedoch an dem Konzept tendenziell festgehalten werden, nicht zuletzt aus Mangel an gangbaren Alternativen. Ob in bestimmten Bereichen – wie z. B. der Allergologie – einfache Standarduntersuchungen die argumentative Vorgehensweise in der Anwendung des Konzepts ergänzen oder gar ersetzen können, ist umstritten.

Man ist sich insgesamt der Probleme und des Handlungsbedarfs sehr bewusst. Die größten Herausforderungen, die es dabei im Hinblick auf eine „Verwissenschaftlichung" dieses Konzepts zu bewältigen gilt, liegen in der Berücksichtigung von möglichen Sekundäreffekten und in der Schaffung von Grundlagen sowie der Präzisierung der Rahmenbedingungen für den Vergleich zwischen transgener und konventioneller Pflanze.

Um mögliche Sekundäreffekte von gentechnischen Veränderungen routinemäßig mittels „Profiling-Techniken" zu detektieren, bedarf es noch intensiver Forschungsanstrengungen, da die Ergebnisse aus solchen Vergleichen auch interpretiert werden müssen. Ferner sind Entwicklungsanstrengungen erforderlich, um zu standardisierten und validierten Untersuchungsmethoden zu kommen. Obwohl innerhalb der EU erhebliche Anstrengungen in dieser Richtung unternommen werden, lässt sich ein baldiger Einsatz solche Techniken nicht absehen. Im Hinblick auf konventionelle Vergleichsorganismen fehlen vor allem die Datengrundlagen, aber auch grundlegende Kenntnisse über den ursächlichen Zusammenhang von Umwelteffekten und Genexpression sowie Pflanzenmetabolismus. In beiden Fällen ist auch die Sammlung von Daten und Erstellung von Datenbanken über Inhaltsstoffe und deren natürliche Variationen erforderlich.

Die weitergehende Verbesserung, Harmonisierung und Konkretisierung der Sicherheitsbewertung von Gentechnikprodukten im allgemeinen und des Konzepts der Substanziellen Äquivalenz im besonderen ist ohne Zweifel von zentraler Bedeutung. Die Gewährleistung eines hohen Sicherheitsstandards ist eine unbedingte Voraussetzung für die weitere Entwicklung, Kommerzialisierung und Diffusion der Gentechnologie und damit ein bestimmender Faktor für die Realisierung des innovatorischen und wirtschaftlichen Potentials.

In diesem Sinne sind Bemühungen von OECD und EuropaBIO zu begrüßen, Konsensus-Dokumente für die Untersuchungen von einzelnen Nutzpflanzen zu formulieren, die z. B. die wesentlichen Inhaltsstoffe und deren Konzentration auflisten. Ebenso sind die Aktivitäten um die Erprobung von „Profiling-Techniken" wichtig, wie sie beispielsweise im Rahmen des ENTRANSFOOD Netzwerks der EU vorangetrieben werden. Zusätzlich gilt es auch, die bisherigen Praxiserfahrungen fruchtbar zu machen, wie dies beispielsweise im Rahmen der oben beschrieben Aktivitäten des UBA Wien und des IFF/IFZ geschieht und in dessen Rahmen auch dieser Workshop stattgefunden hat. Der Workshop leistete damit einen wichtigen Beitrag zur Erstellung der Berichte zu diesen Aktivitäten, die bis Mitte bzw. Ende 2002 vom UBA und IFZ erstellt und den Auftraggebern übermittelt werden sollen.

THE CONCEPT OF SUBSTANTIAL EQUIVALENCE – THE RISE OF A DECISION TOOL

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1 ABSTRACT

This paper identifies some of the main milestones in the development of the concept of substantial equivalence as a decision tool since the early 1990s. Most of these milestones have involved intergovernmental meetings or workshops, which have taken place during the last ten years or so. During this period, the concept has evolved and has been refined, as food safety assessors have accumulated experience. The paper also describes the use of the concept as it is used today to structure a safety assessment. It also notes some current trends that should contribute to a practical understanding of how the concept is applied to food safety assessment.

2 INTRODUCTION

This paper describes the concept of substantial equivalence in relation to the food safety assessment of products of modern biotechnology. It also describes how it has evolved since it was first developed. Much of the experience of the author comes from the work of the Organisation for Economic Co-operation and Development (OECD), an intergovernmental organisation in which representatives of 30 industrialised countries from North America, Europe and the Pacific, as well as the European Commission, meet to co-ordinate and harmonise policies. Work on biotechnology/ biosafety has been underway at the OECD since the mid-1980's. Currently, many of the activities related to food safety are undertaken by Task Force for the Safety of Novel Foods and Feeds, which is made up of individuals nominated by the governments of OECD Member countries. For the most part, they work in ministries or agencies with responsibility for ensuring the safety of products of modern biotechnology including genetically modified foods and feeds.

3 THE DEVELOPMENT OF THE CONCEPT OF SUBSTANTIAL EQUIVALENCE

One of the first important developments came in the report of Joint FAO/WHO Consultation in 1991 entitled *Strategies for assessing the safety of foods produced by biotechnology (FAO/ WHO 1991)*. Amongst other things, this report noted that “The evaluation of a new food should cover both safety and nutritional value. Similar conventional food products should be used as a standard and account will need to be taken of any processing that the food will undergo, as well as the intended use of the food." It went on to state that "Comparative data on the closest conventional counterpart are critically important in the evaluation of a new food, including data on chemical composition and nutritional value.” Although this report did not ex-

plicitly use the phrase, *substantial equivalence*, it did introduce the concept of a comparative approach with a conventional counterpart, which is one of the key elements of the concept of substantial equivalence.

This work was taken up by OECD Member countries at the OECD and further elaborated in a publication, *Safety Evaluation of Foods Derived by Modern Biotechnology: Concepts and Principles (OECD 1993)*. This publication noted that it was "… intended for the use of those involved in carrying out safety evaluations of new foods or food components derived by means of modern biotechnology. It elaborates scientific principles to be considered in making such evaluations, based on a comparison with traditional foods that have a safe history of use."

This document explicitly introduced the concept of substantial equivalence. It noted that 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);
- knowledge of the new product/organism with the new components or trait(s).

It described the principles for the application of substantial equivalence as follows:

- If the new or modified food or food component is determined to be substantially equivalent to an existing food, then further safety or nutritional concerns are expected to be insignificant;
- Such foods, once substantial equivalence has been established, are treated in the same manner as their analogous conventional counterparts;
- Where new foods or classes of new foods or food components are less well-known, the concept of substantial equivalence is more difficult to apply;
- Where a product is determined not to be substantially equivalent, the identified differences should be the focus of further evaluations;
- Where there is no basis for comparison of a new food or food component, the new food should be evaluated on the basis of its own composition and properties.

At the time of the development of this document there had been few, if any, safety assessments of products of modern biotechnology. Despite this, there were a number of case studies within the study including: chymosin derived from *Escherichia coli* K-12; *Bacillus stearothermophilus* alpha-amylase derived from *Bacillus subtilis*; lactic acid bacteria; low erucic acid rapeseed oil (LEAR oil); myco-protein; transgenic tomato; transgenic potato; and transgenic rice. Although these case studies were chosen to illustrate the application of the concept, the document stated that they were not evaluations or regulatory reviews.

A next important publication was entitled *Food Safety Evaluation* (OECD 1996). This document was the result of an OECD workshop comprising experts in food safety assessment from OECD Member countries. This text further elaborated the concept by recognising three situations involving substantial equivalence:

- Where there is substantial equivalence between a new food and a traditional counterpart
- Where there is substantial equivalence except for the inserted trait
- Where substantial equivalence does not exist

Similar findings came in the report of a Joint FAO/ WHO Consultation, Biotechnology and Food Safety (1996). Again, this showed three situations in which there can be safety assessment for:

- Products that are shown to be substantially equivalent to existing foods or food components
- Products that are substantially equivalent to existing foods or food components except for defined differences
- Products that are not substantially equivalent to existing foods or food components.

Further elaboration came in the next Joint FAO/WHO Consultation on Foods Derived from Biotechnology (2000) which was intended, amongst other things, to evaluate experience gained since the previous Consultation. It concluded that "substantial equivalence contributes to a robust safety assessment framework." It also noted that “The concept of substantial equivalence is a key step in the safety assessment process. However, it is not a safety assessment in itself; rather it represents the starting point that is used to structure the safety assessment of a new food relative to its conventional counterpart. This concept is used to identify similarities and differences between the new food and its conventional counterpart. It aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy to date for safety assessment of foods derived from recombinant-DNA plants. The safety assessment carried out in this way does not imply absolute safety of the new product; rather, it focuses on assessing the safety of any identified differences so that the safety of the new product can be considered relative to its comparator. “

The results of this consultation clarified the concept not as safety assessment, but as a comparative approach used as part of a safety assessment.

At about the same in 2000, OECD's Task Force for the Safety of Novel Foods and Feeds prepared a report for the G8 Summit of Heads of State and Government, which included a description of the concept of substantial equivalence, and how it is applied. This report recognised that the concept has continued to evolve in the light of experience, and that this should continue to be the case, by noting that "Food safety assessors should keep the concept of substantial equivalence under review …”

4 CURRENT TRENDS

One of the main products of OECD's Task Force for the Safety of Novel Foods and Feeds is the development of consensus documents. Each of these documents is focused on a major food or feedstuff, for example, documents have been published so far on Low Erucic Rapeseed (OECD, 2001), Soybean (OECD, 2001) and Potato (OECD, 2002). These documents describe the role of the comparative approach, that is, the comparison of a product with one having an acceptable standard of safety. To facilitate this, these documents focus on critical components of foods/ feeds derived from such major crops, which can be compared between modified varieties and non-modified comparators with a history of safe use. For example, the consensus documents include information on key food/ feed nutrients, toxins and allergens as well as anti-nutrients. The continuing development of such documents should contribute to a practical understanding of how the concept can be applied to food safety assessment.

5 CONCLUSIONS

The concept of substantial equivalence has been endorsed on numerous occasions in a number of international fora. At the same time, it has evolved and has been refined as food safety assessors have accumulated experience. It is neither intended as a statement about the safety of genetically modified foods in general, nor as a safety assessment in itself. Rather it is a starting point to structure a safety assessment of a new food relative to a conventional counterpart.

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INTERPRETATION OF SUBSTANTIAL EQUIVALENCE IN THE EU

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Disclaimer: The views expressed in this article are those of the author and do not necessarily represent the views of the European Commission.

1 SUMMARY

Substantial Equivalence, as a scientific concept, has been applied in a consistent way by the relevant Scientific Committees of the European Commission as a tool for the risk assessment of genetically modified plants and novel foods. The interpretation of the concept has developed as science evolved, highlighting the importance of detailed and up-to-date guidance. Such guidelines are being developed and should focus on the needs of the risk assessors, but should also consider elements of risk management in order to render them operational. The use of the concept for legislative purposes led to a different interpretation and is proposed to be abolished in future legislation. The European Food Safety Authority will, in the near future, be responsible for the risk assessment of genetically modified plants and novel foods, and for the development and updating of guidelines covering the interpretation of Substantial Equivalence.

2 INTRODUCTION

The concept of Substantial Equivalence, originally introduced by OECD in 1993 (OECD 1993), was further elaborated by WHO/FAO (WHO/FAO 2000) and experience with its application as a tool in the assessment of the safety of genetically modified and other products has since been gained in Europe and elsewhere. The present text addresses the subject from the European Commission point of view and focuses on the work of the Scientific Committee on Food of the European Commission, and the Scientific Committee on Plants, where relevant. It is recognised nevertheless that considerable experience is also available on the EU national level.

Besides its use in risk assessment, the establishment of Substantial Equivalence has also been applied as a decision criterion in regulating the process of market authorisation of novel foods in Europe.

3 SUBSTANTIAL EQUIVALENCE IN RISK ASSESSMENT

3.1 Role of the Scientific Committees

In the wake of the numerous measures to tackle the BSE crisis, the European Commission also rebuilt the existing structure of scientific advice on the European level in the field of consumer health and food safety. The new structure should enforce the principle of independence of risk assessment from risk management. It comprises a Scientific Steering Committee and eight scientific sector committees (European Commission 1997a) on:

- Food;
- Animal Nutrition;
- Animal Health and Animal Welfare;
- Veterinary Measures Relating to Public Health;
- Plants;
- Cosmetics and Non-Food Products;
- Medicinal Products and Medical Devices;
- Toxicity, Ecotoxicity and the Environment.

3.1.1 Principles of scientific advice

Scientific advice by these committees on matters relating to consumer health is, in the interests of consumers and industry, based on the principles of excellence, independence and transparency. Members of the committees are selected and nominated by the European Commission for three years on the basis of their scientific excellence. They shall act independently from all external influence and shall notify any interests that might be prejudicial to their independence. Members receive no remuneration for their advisory work. The minutes of meetings and the outcome of discussions are published in a transparent manner and are easily accessible.

3.1.2 The Scientific Committee on Food

The Scientific Committee on Food (SCF) was originally established by the European Commission in 1974 (European Commission 1974) to examine problems arising in the context of foodstuffs legislation and relating to the protection of human health and safety of persons. The mandate of the committee was revised in 1997 to comprise

> "…*Scientific and technical questions concerning consumer health and food safety associated with the consumption of food products and in particular questions relating to toxicology and hygiene in the entire food production chain, nutrition, and applications of agrifood technologies, as well as those relating to materials coming into contact with foodstuffs, such as packaging*".

The assessments by the SCF therefore cover a wide range of issues, from chemically well-defined individual substances to complex mixtures, preparations and even whole foods.

The consultation of the committee is mandatory by legislation on many topics related to food safety, such as food additives, food contact materials, flavourings, food hygiene and microbiology, contaminants, nutrition and novel foods.

The Novel Food Regulation (European Commission 1997b), which is relevant in the present context lays down in Article 11 that

> "*The Scientific Committee for Food shall be consulted on any matter falling within the scope of this Regulation likely to have an effect on public health*."

These consultations comprise the assessment of products for which market authorisation is sought, but also questions of general nature. A more detailed description of the role of the SCF under the Novel Food Regulation is given below.

3.2 Interpretation of Substantial Equivalence by the SCF

The Committee addressed the concept of Substantial Equivalence in its guidelines for applicants for market authorisations (SCF 1996a,b,c) and has applied it since mainly for the assessment of dossiers in the context of the Novel Food Regulation. The advice of the SCF was also published by the European Commission as recommendation (European Commission 1997c) to industry and member states' authorities.

3.2.1 The SCF guidelines for Novel Food applications

In introducing the concept the Committee largely made reference to the existing terminology used by OECD, stressing that the establishment of Substantial Equivalence is not a safety or nutritional assessment in itself, but an approach to compare a potential new food with its conventional counterpart. While this principle is widely used mainly in the assessment of genetically modified products the SCF was of the opinion that its application can be extended to the evaluation of all foods from novel sources and processes.

The establishment of Substantial Equivalence is an analytical exercise in the assessment of the relative wholesomeness of a novel food compared to an existing food or food component. In the view of the committee it contains a dynamic element, as the continuing modification of a food requires that the basis of comparison will evolve in a way that the most recent novel food is compared with an approved novel food and not necessarily with the traditional counterpart.

The comparison may be a simple task or be very lengthy depending upon experience with and the nature of the novel food under consideration. According to the SCF Substantial Equivalence may be established for the whole food or food component including the introduced change, or it might be established for the food or food component except for the specific change introduced. In practice, the latter option is consistently used for all genetically modified products evaluated by the SCF.

3.2.2 The safety evaluation of products

For genetically modified products Directive 90/220 was originally the only piece of Community legislation applying to the placing on the market of products containing or consisting of genetically modified organisms and intended for use as foods and food ingredients (Council 1990, European Parliament and Council 2001). Since coming into force of the Novel Food Regulation in 1997 food or food ingredients which contain or consist of genetically modified organisms may not be placed on the market without authorisation under this Regulation. This implies that, even if such a product had been granted market authorisation under Directive 90/220, it may not be placed on the market until it is also authorised under the Novel Food Regulation.

No authorisations under Directive 90/220 were granted since October 1998 and there were 14 applications pending (European Commission 2001a), which have been reduced to 12 recently. This might explain the fact that only for a limited number of genetically modified food products market authorisation has been sought and only a few dossiers were submitted to the SCF for evaluation (tomato products, *Radicchio rosso*, green hearted chicory, GA21 maize, Bt11 maize). For all of these, authorisation under Directive 90/220 is also pending.

Table 1: Status of applications for market approval of genetically modified products under the Novel Food Regulation, October 2001.

Product	Current Status	Substantial equivalence
GM processed Tomatoes	SCF Opinion September 1999. Application withdrawn by company in September 2001.	Established except for introduced traits.
GM *Radicchio rosso* with male sterility	Dossier suspended by company in July 2001. File closed by the SCF.	Additional information on substantial equivalence had been requested.
GM Green hearted Chicory with male sterility	Dossier suspended by company in July 2001. File closed by the SCF.	Additional information on substantial equivalence had been requested.
GM Roundup Ready Maize line GA21	Evaluation finalised. Final adoption by SCF pending.	*Preliminary conclusions: Substantial equivalence established except for the introduced traits.* Extensive toxicity database in relation to traits submitted.
Food products derived from GM insect-protected fresh and processed Bt 11 sweet maize	Pending by SCF, awaiting additional information	Pending.
GM Liberty Link Soybean by AgrEvo	Initial Assessment Report pending, SCF not consulted yet.	–
MaisGard®/Roundup Ready® (GA21 X MON810)	Initial Assessment Report pending, SCF not consulted yet.	–
Crosses between GM maize T25 and MON810 (T25 X MON810)	Initial Assessment Report pending, SCF not consulted yet.	–
Foods and food igredients derived from Roundup Ready® Sugar Beet	Initial Assessment Report pending, SCF not consulted yet.	–
Food products of GM Bt CRY1F Maize Line 1507	Initial Assessment Report pending, SCF not consulted yet.	–
GM High Oleic Soybean Sublines derived from transformation event 260-05	Initial Assessment Report pending, SCF not consulted yet.	–

Only limited conclusions on the interpretation of the concept of Substantial Equivalence in the assessment of individual products can be drawn from this list. The Committee followed the general practice of establishing Substantial Equivalence for the product with the exception of the introduced traits. An extensive database on the toxicology of these traits is then required to assess any potential effects. However, in the case of tomato products, no specific

toxicological information was required, as the introduced gene and the gene product undergo degradation/denaturation during processing. The SCF concluded that:

> *"The present assessment does not cover raw tomato fruit as a novel food or food ingredient. The only tomato products that can obtain clearance from this assessment are those from the hybrids derived from the TGT7F inbred line that have been processed, and that are subject to a heat treatment. This heat treatment causes biological inactivation of the APH(3')II protein and of the npt II and truncated PG genes."* (SCF 1999).

This dossier was also submitted under Directive 90/220 for market approval. It was evaluated by the Scientific Committee on Plants (SCP), which drew similar conclusions:

> *"Heat processing ensures that the enzyme NPTII does not survive in a biologically active form. Regular human consumption of tomato products containing the heat-denatured protein has not caused recognised problems relating to toxicity or allergenicity. Neither effect would have been expected as judged by comparisons of amino acid sequences made with known antigens, the published lack of effects of the intact NPTII protein in chronic toxicity studies in rats and the recorded ease of degradation of this protein in the digestive tract."* (SCP 1998).

It is worthwhile noting that the company has recently withdrawn this dossier from the regulatory process under Directive 90/220 and the Novel Food Regulation for marketing reasons.

More extensive experience has been gained by the SCF in the evaluation of other products than genetically modified ones falling within the scope of the Novel Food Regulation. This includes a wide variety of products considered to be novel for the European market.

Table 2: Status of applications for market approval of non-genetically modified products under the Novel Food Regulation, October 2001.

Product	Current Status	Substantial equivalence
Vit-Enzym.	*No SCF consultation.Application withdrawn March 2000.*	–
Fruit preparations pasteurised using a high pressure treatment process.	*No SCF consultation.* *Commission Decision 2001/424/EEC.*	–
Trehalose	*No SCF consultation. Commission Decision September 2001.*	–
Stevia rebaudiana (plant and dried leaves).	SCF opinion June 1999. Commission Decision 2000/196/EC	Not established. Data insufficient for safety evaluation.
Phospholipids from egg yolk.	SCF opinion June 1999. Commission Decision 2000/195/EC.	Established. No additional data on safety required.
Ngali nuts.	SCF opinion March 2000. Commission Decision 2001/17/EEC.	Not established. Data insufficient for safety evaluation.
Yellow fat spreads with added phytosterol esters.	SCF opinion April 2000. Commission Decision 2000/500/EC.	Not established for ingredient. Extensive database for safety evaluation submitted.
Bacterial Dextran.	SCF opinion October 2000. Commission Decision 2001/122/EEC.	Not established. Extensive database for clinical use available.

Product	Current Status	Substantial equivalence
Soluble and insoluble Fractions of cereal brans.	Pending by SCF, awaiting additional information.	Pending.
Salatrim.	Evaluation finalised. Final adoption by SCF pending.	*Preliminary: Not established. Extensive database submitted.*
Plant sterol enriched Frankfurters, sausage & cold cuts.	Dossier submitted June 2001.	Pending.
Plant sterol enriched bakery products, grain based snack products and gum arabic pastills	Dossier submitted June 2001.	Pending.
Reducol (plant sterol)	SCF not consulted yet.	–
Tahitian Noni Juice	SCF not consulted yet.	–
MCT/Sardine oil structured lipid	SCF not consulted yet. Initial Assessment Report pending.	–
Echium Oil	SCF not consulted yet. Initial Assessment Report pending, additional information requested.	–
Coagulated potato protein and hydrolysates thereof	SCF not consulted yet. Initial Assessment Report pending.	–
Fungal oil	SCF not consulted yet. Initial Assessment Report pending.	–
Gamma-Cyclodextran	SCF not consulted yet. Initial Assessment Report pending.	–
DHA-rich oil	SCF not consulted yet. Initial Assessment Report pending, additional information requested.	–

Also for these products an extensive toxicological database was required if Substantial Equivalence could not be established.

4 SUBSTANTIAL EQUIVALENCE IN LEGISLATION

4.1 The Novel Food Regulation

This Regulation provides a harmonised approval procedure for market authorisation of novel foods. For certain products derogation from this approval procedure is possible based on the establishment of Substantial Equivalence.

4.1.1 Application for authorisation

A company must submit an application for market authorisation to a member state. The authorities evaluate the submitted information and prepare an Initial Assessment Report, which is circulated to all EU Member States for comments or objections within a given deadline. In the absence of reasoned objections, market authorisation is granted. In the case of objections, a Community decision on the authorisation has to be taken, which is commonly based on scientific advice by the SCF concerning the safety of the product.

4.1.2 Notification of placing on the market

In cases where a competent food assessment body confirms that “Substantial Equivalence” to existing foods can be established for a product, simply a notification of placing on the market of certain products is sufficient. At the time of adoption of the Regulation this simplified procedure for market approval was considered to be the best compromise between the need for detailed risk assessments of novel foods and the legitimate interest to reduce the costs of the approval procedure when justified. Substantial Equivalence seemed to provide the appropriate tool to distinguish between the different procedures. A number of food products were granted market authorisation under the notification scheme on the basis of their established Substantial Equivalence.

With increasing experience in the application of this procedure, differences in the interpretation of the concept of Substantial Equivalence became evident. New criteria were introduced, such as the requirement that the establishment of Substantial Equivalence would only be acceptable for products, which do not contain detectable amounts of modified DNA, and/or protein resulting from the genetic modification (European Commission 1998).

In September 2000 a Member State raised doubts about the safety of a number of products, which had been notified previously on the basis of their Substantial Equivalence, and suspended the trade and use of these products by means of invocation of Article 12 of the Novel Food Regulation (European Commission 2000). The safeguard clause laid down in this article also foresees that such a case has to be supported by safety-based grounds. The European Commission consulted the SCF on this case, which concluded that the information presented by this Member State did not provide scientific grounds for safety concerns (SCF 2000).

These examples show that a different interpretation of Substantial Equivalence exists when used in legislation, where it is applied as a tool for risk management. Experience during recent years has highlighted the difficulties in applying a dynamic concept, which is undergoing further refinement and development, as a decision criterion in legislation.

5 FUTURE DEVELOPMENTS

5.1 Revision of guidelines

In order to reflect scientific developments and the experience gained in the application of guidelines, SCF and the SCP are jointly revising their guidelines with a view to establishing consistent guidance for the assessment of GMOs. Although not yet finalised, this activity is at a rather advanced stage. The discussions include for example the principle question of the level of detail necessary for scientific guidance in this field.

5.2 Revision of the legislation

The European Commission has recently presented a proposal for legislation regulating novel food and novel feed, which provides an improved, harmonised and uniform procedure for safety assessment of genetically modified food (European Commission 2001b). A single uniform application procedure without options for derogation is foreseen. Risk assessments of products are generally referred to the future European Food Authority, whose tasks will also include the updating and further development of guidance in this area.

5.3 The European Food (Safety) Authority

As outlined in its White Paper on Food Safety (European Commission 2000b) the European Commission has proposed the establishment of a European Food (Safety) Authority (European Commission 2000c, amended by European Commission 2001c). The legal decision for its establishment has been taken (European Parliament and Council 2002) on 28 January 2002. The core task of the Authority will be to provide independent scientific advice and support on food safety issues and other related matters such as, animal health/welfare, plant health, GMOs and nutrition at the request of the Commission, the European Parliament (EP) and the Member States as a basis for risk management decisions. The Authority will assess risks related to the food chain and give the general public information about food risks. It will also be charged with setting up a network for close co-operation with similar bodies in Member States. The present proposal foresees that the Authority establishes a Scientific Committee and sector-specific scientific panels. Taking into account the multidisciplinary nature of the assessment of genetically modified organisms, and the experience gained with the work of the SCF and the SCP, this area will be covered by one specific scientific panel. The European Commission has recently created a Joint Working Group on GMOs/Novel Food with members of the SCF, SCP and the Scientific Committee on Animal Nutrition to facilitate the evolution of the present system into the future structure to be created by the Authority.

6 CONCLUSIONS

The concept of Substantial Equivalence is applied in a consistent manner in and among the relevant European Commission Scientific Committees. It has been used successfully as one of the tools to assist the formal process of risk assessment of genetically modified food products. Equally, it was found to be applicable to other, not genetically modified products.

It is evident that a clear line must be drawn between the concept of Substantial Equivalence as applied as a tool for risk assessment, and Substantial Equivalence used as a tool for risk

management in the Novel Food Regulation. Legislation has to be applied consistently, uniformly and in a harmonised way in all EU member States, a task which is difficult to achieve by using a dynamic concept the interpretation of which is still under development and refinement.

Substantial Equivalence is established on a case-by-case basis, it is not a tick-box approach. Slight differences in the interpretation over time occur due to progress in the underlying scientific basis, and the experience gained with its application. As it is an analytical approach the application of the concept will also change with the development and refinement of the available methods.

There is a need that these developments in the interpretation of the concept are collated into consolidated and updated guidelines. However, opinions might vary on the level of detail feasible or necessary. Risk assessors in the field of novel foods are used to follow a case-by-case approach, for which broader definitions might be needed. On the other hand, applicants for market approval rightly urge for more explicit guidance on the details necessary for a dossier to comply with the expectations of the assessors, and the procedures followed by them.

Guidelines on the interpretation of Substantial Equivalence will need to be based on a scientific approach and focus on the needs of the risk assessors. In addition, they might have to include elements of risk management to make them acceptable to all stakeholders and render them really operational.

Future developments on the European Commission and EU level are targeted at further increasing consistency and effectiveness of the process of Risk Assessment, which includes the application of Substantial Equivalence. The aim of all efforts in this area should be to improve scientific advice in a way that it can serve as a useful basis for risk management decisions.

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FROM CONCEPT TO PRACTICE: INTERPRETATION OF SUBSTANTIAL EQUIVALENCE IN NORTH AMERICA

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1 ABSTRACT

The regulatory challenge of evaluating the human health and environmental safety of commercial genetically modified crops has been met in Canada and the USA by reliance upon a comparative assessment mechanism (substantial equivalence) that derives from classical plant variety testing. A key difference between the Canadian process and that used elsewhere is an explicit focus in Canada upon novelty in the new crop variety, as opposed to the origins of that novelty. The evaluation mechanisms currently in use appear to have avoided any major safety problems with the first generation of GM crops. However, the complexity of the next generation of GM crops will require more sophisticated assessment methods and tools in order 1) to establish that harmful outcomes are improbable, and 2) to reassure consumers that GM product assessment is capturing all the essential information.

2 INTRODUCTION

The development of plant genetic engineering technology following the discovery of *Agrobacterium*-mediated gene transfer mechanisms in the 1980s was extremely rapid. A few large agrochemical companies recognized already in the early days of the science the commercial potential of creating novel plant genotypes that could be both proprietary products and agents for enhancing the sales of their agricultural chemicals. Based on that potential, they invested heavily in refining the techniques for transfer and control of genes. As they were acquiring ownership of the genetic toolkit, they were also vertically integrating through acquisition of seed companies with established marketing and distribution networks. By the late 1980's, these coordinated investments had led to development of the first GM crop varieties, which were poised to enter commercial production.

The developers of the new GM varieties were eager to introduce into the marketplace the products of their decade of research and development, and thereby begin to recoup that investment within the lifetime of the patents that provided proprietary ownership. However, it was not clear what path they should follow in order to gain the necessary permission. Historically, new crop varieties cannot be marketed in Canada without approval from the appropriate regulatory bodies, primarily the federal ministries, Agriculture and Agri-food Canada (http://www.inspection.gc.ca/english/ppc/biotech/gen/approvale.shtml) and Health Canada (http://www.hc-sc.gc.ca/english/archives/releases/2001/2001_13ebk4.htm), working in cooperation with crop-based expert committees. The regulatory mechanisms existing at that time in Canada, and elsewhere, for approval of new varieties were designed to deal with plant materials derived from classical breeding programs. Thus, the central question facing Canadian regulators, when they were approached by agbiotech companies about their new products, was this:

Could GM crop varieties be accommodated within the existing regulatory framework, or did they require a fundamentally different approach?

If, as the companies maintained, a GM crop represented simply another variation in the spectrum of systematic genetic improvements that had been created in crop species over the last hundred years, then the existing mechanisms would presumably address any relevant issues. These were generally questions of agronomic performance (e.g. yield, disease resistance) and product utility (e.g. oil content, protein quality), which are routinely benchmarked against existing approved varieties. On the other hand, if a genetically engineered crop raised new questions that had traditionally not been addressed in evaluation of new crop varieties, were these questions of sufficient importance to warrant new mechanisms of regulatory assessment?

There is little public documentation of the many discussions that were held around this question within Canada, but they involved numerous government officials, university researchers and representatives of commercial biotechnology firms. On the international front, Canadian regulators and scientists also participated actively in the FAO/WHO meetings, starting in 1990, as well as the related OECD discussions around the issue of GM crop regulation. A useful summary of those international discussions is available at the OECD web-site (http://www1.oecd.org/ehs/service.htm).

The outcome of these on-going deliberations was the evolution of a rational model for Canadian GM crop evaluation, one that is currently unique in the world. In this model, regulatory safety assessment is triggered exclusively by the presence of a “novel trait”, rather than by the method of production of the new crop variety. In other words, regardless of whether the novel trait arises from a wide cross, from mutation breeding or from genetic engineering, Canadian safety assessment focuses on the implications of that novel trait for human and environmental health. Many operational challenges remain, of course, including both the definition of a novel trait and the identification and implementation of appropriate safety assessments for any given trait. The way in which these challenges have been met over the past decade is intimately linked to the use of the concept of “substantial equivalence”.

3 THE ROOTS OF “SUBSTANTIAL EQUIVALENCE”

In everyday life, we typically evaluate any new entity by comparison with a familiar related form. The familiar form thus provides an experience-based reference point. In our modern technology-based society, formal comparisons are made systematically as part of the assessment of a myriad of new products each year, including industrial chemicals, pharmaceuticals, foodstuffs, domesticated animals and crop varieties.

For regulators pondering the need to devise an appropriate mechanism for GMO crop evaluations, the obvious point of reference was the well-established comparative mechanism in use for assessment of new crop varieties derived from classical plant breeding. First, this was an accepted model with a strong infrastructure already in place. Second, the comparative evaluation could, in principle, be relatively straightforward. The GMO genotypes being submitted for scrutiny were derived from existing commercial germplasm, and differed from the parental type only in possessing a very limited amount of new DNA that coded for one or two genes.

Since the products of these genes produced the phenotypic change that comprised the “novel trait”, this did mean that the usual varietal evaluation needed to be extended to include confirmation of the properties of the inserted DNA (e.g. copy number; insert size and integrity), and of its expressed products (mRNA, protein). However, the tools were readily available to obtain those data.

Beyond this specific examination of the transgene and its immediate products, the scope of the assessment of candidate GM varieties has addressed three additional questions:

- Does the GM crop meet performance standards for that crop?
- Does the GM crop pose any risk to the environment?
- Does the GM crop pose any risk to human health?

To a large extent, the structure of the testing used to provide answers to these questions has reflected the methodology used for assessment of varieties derived from classical breeding. The logic for performance evaluation is obvious, since all new varieties must meet producer expectations, regardless of whether they are transgenic. The other two questions, however, are not normally a major priority in variety testing, except in cases where the crop under consideration has a history that raises potential concerns. This is the case, for example, with potatoes, where some genotypes have been found to accumulate toxic glycoalkaloids in their tubers. Since this phenotype is evidently unpredictable, new potato varieties are routinely screened for alkaloid levels.

Overall, the goal of this comparative evaluation was to reassure the evaluators that the GM variety did not differ in any substantial way from the parental variety from which it had been derived, except through possession of the transgene-controlled "novel trait" (e.g. herbicide resistance, insect resistance, etc.). Insofar as it met that criterion, the GM variety could be considered "substantially equivalent" to related, non-transgenic varieties of the same crop. From that baseline, it then had to be established that the transgene and its products posed no risks. Once that had been accomplished, it followed that a GM variety should require no differentiation from non-GM varieties in commercial crop production, during downstream processing, or from the perspective of product labelling.

Neither Health Canada nor the Canadian Food Inspection Agency currently makes any official declaration of "substantial equivalence" in the course of their comparative assessment process, although the term does appear in their published decision trees. In communicating a review decision, Health Canada uses language that reflects its particular mandate, but does not mention "substantial equivalence":

"Health Canada's review of the information presented in support of the food used of Variety X concluded that this variety does not raise concerns related to human food safety. Health Canada is of the opinion that products from Variety X are as safe and nutritious as those available from current varieties"

Nevertheless, the substantial equivalence concept is embedded in the comparative assessment paradigm, since SE represents an entrypoint into the subsequent transgene-focused safety evaluation. Working against that equivalence backdrop, a successful safety evaluation endpoint would be one in which the GM and non-GM varieties would be found to be operationally indistinguishable, **outside of the transgene and its products**. Since 1994, over 45 GM food crop varieties have been found to meet that combination of equivalence and transgene safety in Canada (http://www.hc-sc.gc.ca/food-aliment/english/subjects/novel_foods_and_ingredient/novel_foods_and_ingredient.html).

4 GM vs. CLASSICAL BREEDING – IS THERE A DIFFERENCE?

Plant breeding has been a highly successful process over the past century. In this process, breeders have become very familiar with the agronomic traits of the species with which they work, and with the often complex genetic basis of those traits. They learned that introgression of new qualities to build new genotypes was a slow and uncertain process. Out of the enormous pool of possible allelic combinations generated through crosses, mutation and recombination, only a tiny fraction ultimately prove to represent significant improvements over existing genotypes. Many even perform more poorly. However, a breeder can generally be confident that the progeny of crosses will all retain the broad character of the crop species. In other words, shuffling of alleles within the existing gene pool would not be expected to create any hazardous outcomes, and therefore extensive testing of breeding lines for totally unanticipated hazards has never been carried out.

Genetically more disruptive techniques have also been deployed in plant breeding, however, including wide crosses and mutation breeding. In these cases, genome perturbations would be predicted to be more likely, and in fact the immediate products of these procedures do tend to be defective in many ways. However, they sometimes also generate novel genetic combinations whose phenotypic outcomes make a crop more useful. For this reason, the products of wide crosses and mutation breeding have been incorporated into many breeding programs around the world as part of the overall array of genetic variability from which breeders can make selections.

With respect to varietal assessment, these products have not been subjected to any greater scrutiny than other products of classical breeding. In other words, use of these genetically disruptive procedures has been generally assumed to create no additional hazard in the resulting genotypes, at least once they have been introgressed into other germplasm. Although I am not aware of systematic in-depth studies that have tested this assumption, the weight of experience over the last six decades of scientific plant breeding suggests that it is a valid premise.

This experience has, I believe, formed an important element in the attitude of regulators toward genetically engineered genotypes. There can be little argument that direct insertion of a new genetic element (e.g. a T-DNA construct) into an existing genome represents a disruption event. However, the scale of that disruption is markedly smaller than that induced by ionizing radiation, or by saturation chemical mutagenesis, both of which have been accepted as sources of useful genetic variability. In view of that, it may seem surprising that a T-DNA insertion attracts greater regulatory attention.

This separate treatment arises from the recognition that genetic engineering events are fundamentally different from those produced by other disruptive techniques. Rather than deleting a genetic capability, or re-arranging existing genetic circuitry, GM events are designed to create a new, highly functional genetic element that adds a novel metabolic/physiological capability to the existing cellular machinery. This description applies particularly to the first generation GM crop varieties, where single genes from non-plant sources (e.g. bacteria, viruses) have been inserted into crop genomes.

In Canada and the USA, regulatory oversight is automatically triggered by the presence of the novel trait created by such GM events, although for different reasons.

It is important to note that within the Canadian regulatory model, the trigger for assessment is the novelty, rather than the method of development of that novelty. In principle, this means that, in Canada, a particular GM variety can be regarded as lacking novelty and therefore requiring no evaluation. This might be the case, for example, for a transgenic variety derived from an earlier transgenic variety of the same crop through further crosses with other breeding lines. The progeny still carry the same, previously evaluated transgene in a very similar genetic

background, and could therefore be considered to present no new risks. On the other hand, a new variety derived through classical breeding, and displaying an unusual (but agronomically valuable) trait, would be subject to regulatory evaluation.

By contrast, in the USA, all crop varieties carrying transgenes are presently subject to evaluation, no matter what their provenance, whereas the products of other disruptive techniques (e.g. mutation breeding) are exempt. This distinction between the two systems is based on the Canadian view that the requirement for health and environmental evaluations should be driven by the potential for risk, no matter what its source.

In the Canadian system, Health Canada regulators have focussed, understandably, on potential health hazards directly associated with the transgene product. For example, if a modified EPSP synthase enzyme is being expressed at high levels in the plant, is this protein toxic to consumers of the plant, or is it possibly allergenic? The Canadian Food Inspection Agency would be more concerned with the environmental impacts of the transgene and its product, including gene transfer to other organisms. Much of the CFIA evaluation revolves around field trial testing carried out over a number of years, using a modified model derived from traditional variety testing procedures.

In the USA, a somewhat different distribution of responsibilities is in place, involving the EPA, FDA and the USDA, but the evaluation paradigm is essentially the same. It is worth noting, however, that the FDA has only recently announced its intention to introduce a mandatory pre-market notification scheme like Canada's. Thus far it has relied upon a voluntary consultation process, although this has been scrupulously observed by industry. Despite the strong similarity in the approaches used in Canada and the USA, only in 1999 was any formal attempt made to begin harmonizing the two systems (http://www.inspection.gc.ca/english/plaveg/pbo/usda05e.shtml). Since 1994, Canada has, however, accepted the molecular data generated in support of a submission in the USA to support a parallel Canadian submission.

At both Health Canada and CFIA, the possibility that secondary changes may have been induced in the plant as a result of interactions between the transgene and/or transgene products and the rest of the cellular machinery, has always been recognized. However, the operating assumption has been that these secondary effects would probably be minor, and that any consequences for safety would, in all likelihood, be identified in the course of the food safety and environmental safety assessments. On one level, the experience of plant breeders over time with other genome-disrupting techniques lends credibility to this assumption. On another level, however, it is important to recognize that the consequences of overlaying an existing plant genome with completely novel functions are not well understood. Even based on our limited knowledge of the complexity of cellular organization and regulation, it is unlikely that the pleiotropic impacts associated with such an overlay can be realistically predicted by extrapolation from our experience with mutation breeding. Our current inability to predict with confidence what might have been altered in the phenotypic background of a GM plant thus places a large burden on the regulatory process, since it should, in principle, be capable of detecting and assessing essentially all possible outcomes. In the context of a comparative assessment, this makes the establishment of equivalence with the non-transgenic benchmark a truly daunting task.

5 THE WAY FORWARD

The relative safety of mutation breeding as a source of plant genetic variation was only established over many years of experience with use of its products in commercial varieties. This empirical approach was followed because there were no tools available that could report the genetic or physiological status of the mutated plant in any detail. Today the situation has changed. With the recent development of global profiling technologies, it is now possible to measure changes in gene expression, protein accumulation, protein post-translational modification patterns and metabolite pools (Oda *et al*, 1999; Schenk *et al*, 2000; Natera *et al*, 2000). Not only can the full extent of any changes induced by transgene insertion be monitored but, equally important, the range of natural variation in these same genetic and physiological traits can be assessed across environments and time. It would be crucial to establish the "phenotypic envelope" for a given crop using these tools, so that the significance of any deviations can be established by correlation with the results of the environmental and human health impact studies.

Adoption of global profiling techniques as a central component of GM variety assessment would greatly strengthen the current mechanism of comparative evaluation. By enriching the panel of criteria used to establish "substantial equivalence" the evaluation process itself will gain considerable credibility. At the same time, the rapid accumulation of correlative data that link molecular profiles to utility and ecological performance will make it possible in the future to predict with increasing confidence the probable biological consequences of specific genetic alterations. In the long term, this predictive capability has the potential to simplify the entire process of variety assessment, no matter what the origins of the genetic changes that have been introduced.

The alternative approach is, in effect, to deploy GM varieties for many years and watch for any negative consequences. This would emulate the long period of use of mutation breeding and may very well reach a similar conclusion as to the safety of GM products. However, it seems unlikely that this "wait and see" scenario would be tolerated by consumers, many of whom are insisting on more reassurances from their regulatory system, and more transparency in the science-based process by which regulatory decisions are reached. If the commercial deployment of GM technology is to continue, therefore, it will need to be supported by a comparative assessment regime that brings more convincing data to the table in establishing "substantial equivalence" and product safety.

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THE LIMITATIONS AND POTENTIAL UTILITY OF SUBSTANTIAL EQUIVALENCE

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1 ABSTRACT

It has recently become increasingly clear that the concept of 'substantial equivalence' could be interpreted as either a '*decision threshold*' or as a '*safety standard*'. The latter interpretation may have much to commend itself, but the former is seriously problematic. Moreover, in practice the concept has been widely used as a decision threshold while masquerading as if it were a safety standard. Recently, there has been a growing recognition of the shortcoming of the ways in which 'substantial equivalence' has been used, and the ensuing debates have culminated in the recent proposal from the European Commission to abandon using the concept. While that proposal may be sensible and welcome, there remains a need to develop an alternative approach to put in its place. A systematic process should be initiated which will involve consultation with representatives of a wide range of scientific disciplines and professions to develop an alternative approach. That process should seek to establish the conditions under which a broad range of disciplines and investigative methods could provide useful information about the putative safety of GM foods. Ultimately, however, the decisions about how much, and which kinds of, evidence, tests and data are required can never be purely scientific judgements. The institutions and processes by which those decisions get taken need to be informed by scientific advice, but ultimately their legitimation must be democratic rather than scientific.

2 INTRODUCTION AND CONTEXT

I published, in *Nature,* in October 1999 a critique of the meaning and use of the concept of substantial equivalence. In that paper, I characterised its use as unscientific, pseudo-scientific and anti-scientific.[1] My co-authors and I argued that *"Substantial equivalence is … anti-scientific because it was created primarily to provide an excuse for not requiring biochemical or toxicological tests. It therefore serves to discourage and inhibit potentially informative scientific research."*

The concept of substantial equivalence was first introduced in 1993 by the OECD, saying:

> *"The concept of substantial equivalence embodies the idea that existing organisms used as food, or as a source of food, can be used as the basis for comparison when assessing the safety of human consumption of a food or food component that has been modified or is new."*[2]

[1] E Millstone, E Brunner and S Mayer, 'Beyond the "substantial equivalence" of GM foods', *Nature*, Vol 401, 7 October 1999, pp. 525-526

[2] OECD, *Safety evaluation of foods derived by modern biotechnology*, Paris, 1993, p. 11

3 CLARIFICATION OF MY ARGUMENT

My arguments have been misunderstood in at least two key respects. If I failed to make myself sufficiently clear, I apologise. My critique of 'substantial equivalence' was entirely directed against the ways in which the concept was being used, I was not arguing that there never could be a legitimate usage for that term. Since my paper was published, several teams have tried to articulate what might be a proper use for the concept, and I welcome their contributions to the debate. A second respect in which my argument was misunderstood was that it was interpreted as suggesting that all GM foods, but no non-GM foods, should be tested to exhaustion by every conceivable means. That has never been my view, as I will explain below.

It is conceivable that the concept of substantial equivalence could have served, and might yet serve, as a hypothesis guiding further research, but that is not the way in which it has been used by official risk assessors and by risk-management policy-makers. Instead of using it as a **preliminary hypothesis** to be tested using a range of toxicological and immunological tests, it has been used as grounds for not requiring the conduct of those potentially relevant tests. As an FAO/WHO panel has acknowledged, a decision to deem some GM food to be 'substantially equivalent' to some conventional counterpart has been tantamount to a decision not to require the conduct of toxicological and immunological tests.[3]

Earlier this year, the Canadian Royal Society elaborated the contrast between a 'decision threshold' and a preliminary hypothesis that might provide a safety standard. The Canadian team identified:

> *"…two different uses of the concept of 'substantial equivalence':*
>
> 1. *A GM organism is 'substantially equivalent' if, on the basis of reasoning analogous to that used in the assessment of varieties derived through conventional breeding, it is assumed that no changes have been introduced into the organism other than those directly attributable to the novel gene. If the latter are demonstrated to be harmless, the GM organism is predicted to have no greater adverse impacts upon health or environment than its traditional counterpart. We refer to this interpretation as the* decision threshold *interpretation.*
> 2. *A GM organism is 'substantially 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. We refer to this interpretation as the* safety standard *interpretation."*[4]

The Canadian Royal Society "… accepts the validity of the concept when used in the 'safety standard' interpretation ... [but has] … grave reservations about its validity when employed in the 'decision threshold' interpretation."[5] I am at one with the Canadians on that point, yet all the evidence overwhelming indicates that the concept has been used as a decision threshold while masquerading as if it were a safety standard.

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[3] Joint FAO/WHO Food Standard Programme Expert Consultation, *Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology*, 14-17 March 2000, Document CX/FBT/00/2 Appendix 2 to Annex 1, p. 13, para 7

[4] Canadian Royal Society, Elements of Precaution: Recommendations for the Regulation of Food Biotechnology in Canada, an Expert Panel Report on the Future of Food Biotechnology prepared by The Royal Society of Canada at the request of Health Canada, Canadian Food Inspection Agency and Environment Canada, February 2001, Ch 7, pp. 193-4

[5] Canadian Royal Society, February 2001, p. 183

4 HOW THE CONCEPT OF SUBSTANTIAL EQUIVALENCE HAS BEEN USED

Evidence that 'substantial equivalence' is not a robust scientific judgement but one that is officially contested has recently been provided in two reports, one by Friends of the Earth and the other by a Dutch team from the Schenkelaars Biotechnology Consultancy. Friends of the Earth systematically tabulated the differences in the judgements made between US and EU regulatory authorities in respect of 10 sets of decisions, concerning 3 GM varieties of oilseed rape (canola) 6 of maize and one potato.[6] In each case conflicting judgements had been made, as between the US government and at least one EU Member State and/or the European Commission. If ascriptions of substantial equivalence were robust scientific judgements then the differences they located should not have occurred.

The Schenkelaars report compared judgements within the EU, and revealed just how little agreement there has been about how the term 'substantial equivalence' should be used, and when it should be applied.[7] They reported that "…there had been a lack of consistency from case to case in the data provided, even within the same crop species."[8] They also observed that, in relation to GM maize: "…in all these cases of notifications differences in the composition of the GM maize plant and its non-GM control have been observed…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…It is hardly plausible that the compositional data have been analysed in a statistically sound way."[9] They found very wide differences concerning the kinds of information different Member States deemed necessary and/or sufficient for the attribution of 'substantial equivalence'. They also found that in practice far less data was actually required that might have been expected.[10]

Schenkelaars also found that "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."[11]

The gap between using substantial equivalence as a decision threshold rather than as a preliminary hypothesis serving as a safety standard is evident from contrast between the kinds of evidence which have been deemed sufficient, and the kinds of evidence that might properly suffice. Attributions of substantial equivalence have thus far been made primarily by reference only to data from analyses of the chemical composition of GM foods.[12] Those chemical data have, however, been treated as if they provided adequate grounds for secure biochemical, toxicological and immunological extrapolations. The absence of evidence of gross chemical differences between a GM food and a non-GM comparator does not, however, justify the conclusion that they are toxicologically or immunologically indistinguishable. This ex-

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[6] E Diamand, The Great Food Gamble – an assessment of genetically modified food safety, Friends of the Earth, Leeds, June 200, p. 26; available at http://intranet.foe.co.uk/resource/reports/great_food_gamble.pdf

[7] Schenkelaars Biotechnology Consultancy, GM food crops and application of substantial equivalence in the European Union, The Netherlands, June 2001, Commissioned by the Dutch Foundation on 'Consument & Biotechnologie'

[8] Schenkelaars, June 2001, p 5

[9] Schenkelaars, 2001, p. 5

[10] chenkelaars, 2001, p. 15

[11] ibid.

[12] op cit p 3

emplifies the general rule that *the absence of evidence of risk* does not amount to *evidence of the absence of risk*, especially if you fail to gather much of the potentially relevant evidence.

GM crops are slightly tricky to categorize in this context since they are, by definition and by design, genetically and therefore chemically different from their antecedents. An important question for risk assessors and risk managers is how accurately can we characterize those differences, and do they matter? Chemical analyses, by themselves, can give only a very partial and incomplete account that can provide at best a rather poor basis for reaching conclusions about the likely toxicological and immunological consequences of consuming those GM foods.

There is a risk that current techniques for genetic modification could cause unintended and unanticipated changes in the phenotype of the novel organism – known as pleiotropic effects. While many such effects might be noticed and eliminated, some unintended and undesirable changes might be missed. Toxicant, allergens and anti-nutrients might be present yet might not be detected, especially if chemical analyses focussed narrowly on known and anticipated compounds.[13] Consequently, unanticipated changes could modify the occurrence of toxins, allergens or anti-nutrients that would not be detected by a conventional scrutiny of chemical composition. A Dutch government team has acknowledged, for example, that "…compositional analysis...as a screening method for unintended effects...of the genetic modification has its limitations...in particular regarding unknown anti-nutrients and natural toxins…"[14]

Toxicologists have long aspired to possess reliable ways of predicting the toxicological and biochemical activity of chemicals from knowledge of their chemical structures. Predicting the toxicological and immunological consequences of ingesting GM foods from data produced by chemical analyses is considerably more problematic than predicting the chemical activity of a single compound from a knowledge of its chemical formula and structure. To ascribe substantial equivalence to a GM food by reference to data from chemical analyses as if they provided an adequate substitute for a broad range of toxicological and immunological data is not good science.

Ascriptions of substantial equivalence can only become robust scientific judgements if the evidence necessary to support the hypothesis has been generated and analysed. In the interim, its use has been unscientific because it has been misleadingly portrayed as if it were a robust conclusion rather than a preliminary guess. In so far as the ascription of substantial equivalence serves as a disincentive that discourages and inhibits the conduct of a broad range of biochemical, toxicological and immunological studies that would be needed empirically to test that ascription, its use has anti-scientific consequences. I entirely agree with Schenkelaars that differences between GM and non-GM comparators "… should … be the focus of further nutritional, toxicological and immunological evaluation."[15]

[13] UK Medical Research Council, *Report of a MRC expert group on genetically modified (GM) foods*, MRC, June 2000; P Kearns and P Mayers, 'Substantial equivalence is a useful tool', *Nature* 1999, 401: 640

[14] H A Kuiper et al, Food Safety Evaluation of Genetically Modified Foods as a Basis for Market Introduction, 1998, Ministry of Economic Affairs, The Hague

[15] Schenkelaars Biotechnology Consultancy, p. 6

5 RECENT DEBATES ABOUT SUBSTANTIAL EQUIVALENCE

A Joint FAO/WHO Expert Consultation on Biotechnology and Food Safety was held in 1996 and concluded that: "When substantial equivalence is established for an organism or food product, it is regarded to be as safe as its conventional counterpart and *no further safety consideration is needed*."[16] (emphasis added) and that view was reiterated by Codex in 2000.[17] Those remarks were widely interpreted as implying that no further testing or deliberation was required. Four years later, however, the OECD asserted the contrary namely that "Substantial equivalence is not a substitute for a safety assessment."[18] Those contrasting assertions represent at attempt by the OECD, and its Member States, to retreat from the claim that the ascription of 'substantial equivalence' terminates discussions about safety to the suggestion that it might leave safety issues unresolved. The Royal Society of Canada however was not convinced. Its Panel said that: "… application of this term [substantial equivalence] to a new GM variety has become, within the present regulatory environment, effectively a declaration of safety."[19] Similarly the UK's Medical Research Council's report on GM Foods accepted that in practice "Where a food can be shown to be substantially equivalent it is considered to be as safe as its counterpart and no further safety assessment is required."[20] The MRC also acknowledged that "The amount of comparative data required to establish substantial equivalence involves a somewhat subjective judgement."[21]

By February 2000, following the debates about the use of the concept of 'substantial equivalence' and its lack of clarity, the joint FAO-WHO CODEX Committee on Food Labelling decided to omit the term 'substantial equivalence' from its draft recommendations for food and food ingredients obtained through modern biotechnology. As the Canadian Report explained: "This commission had already made the decision to delete the word 'substantial' in 1999, and in 2000, proposed to use such phrases as 'no longer equivalent' or 'differs significantly' in the text of its recommendations. It was suggested that "if the nutritional value of a food or food ingredient is no longer equivalent to the corresponding food or food ingredient", certain conditions would apply, such as informing the consumer of a changed nutrient content. However, this negative approach to "equivalence" appears to constitute a rejection of the concept of "substantial equivalence" altogether, rather than a redefinition of it."[22]

In June 2000, Jose Domingo, a medical toxicologist in Spain published a letter in Science pointing out that exhaustive searches of both the Medline and Toxline databases and found just eight published reports in the peer reviewed literature of studies into the putative toxicology of GM foods.[23] Of those eight, one was the contentious work by Pusztai et al, two others were published in Russian, leaving only five others. Domingo assumed that numerous other

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[16] FAO, 1996, "Biotechnology and Food Safety – Report of the Joint FAO/WHO Consultation, Rome Italy, 30 September – 4 October 1996," FAO Food and Nutrition Paper 61, Rome

[17] Codex Alimentarius Joint FAO/WHO Food Standard Programme, *Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology*, 14-17- March 2000, Document CX/FBT/00/2 Appendix 2 to Appendix 1, p. 13, para 7

[18] OECD, *Substantial Equivalence and the safety assessment of GM foods*, April 2000, available (October 2001) at http://www.oecd.org/subject/biotech/conceptsub.pdf

[19] Canadian Royal Society, Elements of Precaution: Recommendations for the Regulation of Food Biotechnology in Canada, an Expert Panel Report on the Future of Food Biotechnology prepared by The Royal Society of Canada at the request of Health Canada, Canadian Food Inspection Agency and Environment Canada, February 2001, Chapter 7, p. 177

[20] Medical Research Council, Report of a MRC Expert Group on Genetically Modified (GM) Foods, June 2000, available at www.mrc.ac.uk/PDFs/GM_foods.pdf Section 3. p. 11

[21] op. cit. Section 3. p. 11

[22] Canadian Royal Society, *Elements of Precaution*, 2001, Chapter 7, p. 180

[23] J L Domingo, 'Health Risks of GM Foods: Many Opinions but Few Data', *Science*, Volume 288, Number 5472, Issue of 9 Jun 2000, pp. 1748-1749

studies had been conducted, yet their results remained unpublished.[24] My interpretation is rather different, namely that they have not been conducted, because there is neither a requirement nor any incentive for the companies to invest in such work. It might after all provide unwelcome findings.

The MRC's report used characteristically diplomatic language, but as we say in England 'damned them with faint praise'. "The Group agreed that current regulatory procedures, using the principle of substantial equivalence, addressed the theoretically possible health risks of *known* toxins and allergens in GM foods. In the future, the issue of non-intended effects might be aided by new molecular methods to enhance the quality of the data used in the regulatory process. The Group recommended that mechanisms of food allergy should be the subject of further research. This would facilitate the design and development of novel approaches for the identification and characterisation of potential protein allergens."[25]

They also concluded, however that "Most of the theoretical health risks presented by GM foods are addressed in current regulatory assessments; where unresolved issues remain this is principally due to a lack of evidence either supporting or refuting proposed and specific health effects. To address these issues, **a multidisciplinary research strategy is required** which aims **to identify** and quantify any **health risks** that do exist **and to inform future developments in regulatory procedures**."[26] (emphases added)

The report from the Royal Society of Canada similarly recommended: "… a four-stage diagnostic assessment of transgenic crops and foods that would replace current regulatory reliance upon 'substantial equivalence' as a decision threshold."[27]

Specifically, the Canadians recommended: "… that approval of new transgenic organisms … should be based on rigorous scientific assessment of their potential for causing harm to the environment or to human health. Such testing should replace the current regulatory reliance on 'substantial equivalence' as a decision threshold."[28]

In response to those assertions and debates, some official commentators have recently taken to acknowledging that the ascription of substantial equivalence does not constitute a proper risk assessment, but they have taken to asserting that substantial equivalence is being used to 'structure' risk assessments.[29] The invocation of that term suggests that substantial equivalence provides structure to what would otherwise be an amorphous process, but that is misleading. The concept of substantial equivalence is being used rather to truncate and shortcut the process of assessing risk. By reference to a relatively modest amount of data from chemical analyses, wide inferential leaps have been taken, without adequate evidence, and with the consequence that the appropriate evidence was not required or generated. If that is a way of structuring a risk assessment, it is hardly a robust one.

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[24] ibid.

[25] UK Medical Research Council, *Report of a MRC expert group on genetically modified (GM) foods*, MRC, June 2000, p. 1 paras 4-5

[26] UK Medical Research Council, Report of a MRC expert group on genetically modified (GM) foods, MRC, June 2000, p. 2 para 8

[27] Canadian Royal Society, Elements of Precaution: Recommendations for the Regulation of Food Biotechnology in Canada, an Expert Panel Report on the Future of Food Biotechnology prepared by The Royal Society of Canada at the request of Health Canada, Canadian Food Inspection Agency and Environment Canada, February 2001, Executive Summary, p. ix, available at http://www.rsc.ca/foodbiotechnology/indexEN.html

[28] op. cit., Recommendation 7.1, p. x

[29] See eg *Safety aspects of genetically modified foods of plant origin*, Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, World Health Organization, Geneva, 29 May – 2 June 2000, para 4.1, p. 4; and J Bainbridge to Royal Society of London, May 2001, available at http://www.royalsoc.ac.uk/templates/search/websearch.cfm?mainpage=/policy/cur_proj.htm and download gm_sub.pdf

6 RECENT DEVELOPMENTS

The European Commission's July 2001 Proposal for a Regulation on Genetically Modified Food and Feed represents a major development. It said: "In order to ensure clarity, transparency and a harmonized framework for authorization of genetically modified food, this proposal **does not include a** notification (**simplified**) **procedure** as laid down in Regulation EC 258/97 **on novel foods … which are substantially equivalent** to existing foods. "[30] (emphasis added) The draft Regulation stipulates in paragraph 6 that: "In order to ensure clarity, transparency and a harmonised framework this notification procedures [..involving **the attribution of substantial equivalence …] should be abandoned** in respect of genetically modified foods."[31]

Whether that represents an acknowledgement that the way in which the concept has been used has been fundamentally flawed, or whether it is merely an admission that its continued use is no longer democratically acceptable to EU citizens is hard to determine.

7 ALTERNATIVES TO SUBSTANTIAL EQUIVALENCE

While that policy development may be welcomed, it leaves us with a major lacuna. If we discontinue the use of substantial equivalence as a decision threshold, by reference to what evidential bases should judgements about the safety and acceptability of GM foods be decided? To say, as CODEX and others have, that we should continue to rely on substantial equivalence because no-one has yet articulated a plausible alternative is unsatisfactory.[32] The need to develop alternatives to substantial equivalence is surprisingly widely recognised.

Indications that some alternatives are being considered have emerged in the context of a discussion of the imminent arrival of requests for authorization to market GM foods that will have multiple or so-called 'stacked' genetic modifications. Even enthusiastic defenders of the current ways of using the concept of substantial equivalence recognise that it will not provide an adequate basis for evaluating the putative risks of GM foods with multiple modifications.[33] The scope for unanticipated interactions amongst genes that have not hitherto co-existed in any individual species is likely to be greater than with single modifications. There have, consequently, been calls to develop alternative and more discriminating and nuanced approaches to these more challenging innovations. If, however, such alternatives can be developed, then the claim that there is no alternative to substantial equivalence is refuted, and the question arises as to why a more discriminating and nuanced approach could not, and should not, be applied to the relatively simpler constructs?

Many of those who have acknowledged the limitations of the current ways of ascribing substantial equivalence have suggested that improved chemical analyses might diminish the uncertainties and our ignorance. Others acknowledge that more refined chemical analyses might be useful, but also understand that analytical data on their own will never be sufficient, unless we also understand the toxicological and immunological implications of those analyses.

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[30] The European Commission, Proposal for a Regulation on Genetically Modified Food and Feed, 25 July 2001, p. 7

[31] op. cit., Para 6, p. 12

[32] WHO, *Safety Aspects of Genetically Modified Foods of Plant* Origin, Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, June 2000, Geneva, Section 4.4 pp. 7-8, available at http://www.codexalimentarius.net/biotech/en/Answers.htm, and J Bainbridge to Royal Society of London, May 2001

[33] J Bainbridge to Royal Society of London, May 2001

For example the MRC said: "In the future, it is possible that substantial equivalence testing and particularly the issue of non-intended effects might be made more robust by the application of modern molecular approaches. Microarray technology allows the expression of many thousands of mRNA molecules to be screened in a single experiment. Total protein content can also be analysed using two dimensional gel electrophoresis with quantitative image analysis of individual protein spots. Metabolic profiling is another useful approach that might be used to enhance the quality of the data that is examined in the regulatory assessment."[34]

While it may be important to acknowledge the potential contribution of new technologies, such as those arising from advances in genomics and proteomics, many of those advances involve improvements to the techniques of chemical analysis and biochemical characterization rather than to improving our ability to forecast the implications of the revealed differences for public health.

Schenkelaars suggest that "Further development and validation of profiling techniques based on genomics, proteomics and metabolomics may increase the potential to detect unintended differences."[35] But what is significant in that comment is that it provides an acknowledgment that such techniques will require not just development but also *validation*, and also that it may be necessary not just to sequence genes, and chemically to identify proteins but also to understand how they are metabolised; but that is rather like saying that when toxicology is a complete science, we will have fewer problems.

The debate about the utility and limitation of substantial equivalence is complicated by the fact that on different occasions we have been told both that there are no alternatives to substantial equivalence and that alternatives to substantial equivalence have to be developed. The argument that there are no alternatives to using substantial equivalence typically emerges in the context of an insistence that traditional toxicological protocols that are applied to testing single chemical entities, such as the active ingredients in pharmaceutical and pesticide products or food additives, are inappropriate for testing GM foods.

There are problems with the direct extension of those protocols to GM foods, because chronic toxicological feeding studies typically involve obliging experimental animals to ingest substantially greater doses of the test material than humans are ever likely to encounter in normal usage so as to identify a 'maximum tolerated dose' and a 'no adverse effect' level from which an 'acceptable daily intake' can be inferred. On the other hand, there is something perverse in arguing that far less testing is appropriate to a food ingredient that might be consumed in gramme or even kilogramme quantities on a daily basis than is appropriate for a compound that is likely be to be consumed only in milligramme or even microgramme doses, and sometimes for only relatively brief periods.

On the other hand, just because GM foods cannot be examined toxicologically *just like* single chemical compounds, it does not mean that toxicological tests can be neither possible nor desirable nor illuminating. Comparing GM foods toxicologically with their non-GM counterparts can be a useful part of a systematic strategy. It might be relatively straightforward and informative to conduct animal feeding studies in which, for example, the animals' diet included some soybean-derived material or some maize flour and oil. A control group could receive a diet that contained no GM material, while test groups might correspondingly receive diets containing a GM-ingredients at low, medium and high doses. The animals could be monitored for a wide range of *in vivo* and *post mortem* parameters. Moreover, GM crop-derived food ingredients could be tested against *in vitro* tissue culture systems and bacterial mutagenicity systems. The data derived from those studies would not necessarily be harder to interpret than those derived from orthodox chemical toxicology.

[34] MRC 2000 Section 3.3

[35] Schenkelaars, p. 3

As the Royal Society of Canada argued:

> *"The obvious approach to analysis of the consequences of the presence of the transgene is to employ direct testing for harmful outcomes. In the case of food or feed products, this would mean testing for short-term and long-term human toxicity, allergenicity or other health effects. The environmental impacts of both local and landscape-scale deployment of the transgenic organism would also be assessed, over time and across relevant sites. At the end of this comparative analysis, an assessment must be made of the extent to which the transgenic variety deviates from the parental genotype, and whether any observed deviations are biologically significant …This approach has the obvious merit of directly addressing the potential for harm, which is the primary motivation for the regulatory process, and from that perspective it must remain the cornerstone of the approval process."*[36]

Neither the Canadian Royal Society nor I have argued that a massive panoply of toxicological tests should always be conducted for each and every GM food innovation, although my position has been misrepresented in those terms.[37] Whenever animal feeding trials with GM foods are conducted looking, for example, at feed conversion efficiency but not at animal health, those are lost opportunities for more research. I am arguing, rather, that judgements about which studies should be conducted, and which kinds of evidence might be necessary in any particular case, needs to be made on the basis of an empirically rich rather than on a relatively impoverished basis.

The UK government, governments of other EU Member States and the European Commission have so far taken only a few preliminary steps towards exploring the different ways in which various scientific disciplines and groups of practitioners might be able to contribute to the development of more informative and reliable ways of investigating the potential immunological and toxicological effects of GM foods. A few partial studies have been commissioned, but there is so far no evidence of any systematic approach to assembling the fragments together into a coherent and systematic approach.[38]

8 A POSSIBLE WAY FORWARD?

In trying to develop an alternative to current ways of deploying 'substantial equivalence' I am not, however in favour of replacing one simplistic formula with another that is only marginally less simplistic. Risk assessment and risk management policy-making for GM foods needs to be organised on a different basis from hitherto. That basis should be one that encourages rather than inhibits the advancement of scientific knowledge of nutrition, toxicology, immunology and related health disciplines. Such a basis is far more likely to command (and warrant) public confidence than those that has already been discredited.

A systematic process could and should be initiated which would involve consultation with representatives of a wide range of scientific disciplines and professions to develop an alternative approach to substantial equivalence. That process should seek to establish the conditions under which a broad range of disciplines and investigative methods could provide useful information about the putative safety of GM foods. Information of those types could become

[36] Canadian Royal Society, *Elements of Precaution*, February 2001, p. 186

[37] N Smith et al, House Subcommittee on Basic Research, Report to the Committee on Science, *Seeds of Opportunity: An Assessment of the Benefits, Safety, and Oversight of Plant Genomics and Agricultural Biotechnology*, for 160th Congress, 13 April 2000, p. 52, available at www.house.gov/science/smithreport041300.pdf

[38] See eg http://www.biosociety.dms.it/Contracts/KA1/01182.shtm; http://www.ncl.ac.uk/bns/research/res_contracts/res_hgilb7.htm

integral feature of risk assessments of novel foods. The kinds of test that can and should be conducted remain to be examined and decided and a process by which the scientific community could collectively contribute to, and illuminate, our understanding of what tests might be possible, useful and desirable needs to be initiated. This is precisely the kind of process which could most usefully be conducted at a multi-national level, for example under the auspices of the European Commission's DG-SANCO, or at a global level under the auspices of the World Health Organisation.

Ultimately, however, the decisions about how much, and which kinds of, evidence, tests and data are required can never be purely scientific judgements. Science can tell us what kinds of data we might choose to examine, but judgements about how much is sufficient are non-scientific social judgements. Even though, for many risk issues, it has been traditional practice to leave those decisions to scientific advisors, that practice has lost its credibility and legitimacy. The institutions and processes by which those decisions get taken need to be informed by scientific advice, but ultimately their legitimation must be democratic rather than scientific.

VIEWS ON SUBSTANTIAL EQUIVALENCE FROM AN AGRICULTURAL COMPANY

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***Alain Toppan**, Biogemma, France*

Using a few examples, we will try to give you the views on substantial equivalence from an agricultural company.

The substantial equivalence is the key concept of the evaluation of the safety of novel food coming from biotechnologies. This concept is part of the safety assessment framework, by comparison of the new food with appropriate conventional counterpart.

The interest of biotechnology has been underlined several times. “Recognizing that modern biotechnology has great potential for human well being if developed and used with adequate safety measures for the environment and human health” is written in the Cartagena protocol on biosafety, result of years-long discussions between administrations, NGOs and industrials. The FAO report for the year 2000 notes: “Biotechnology provides powerful tools for the sustainable agriculture, fisheries and forestry, as well as the food industry”.

Our Group has also understood the interest of biotechnology from long time and chosen to invest in plant biotechnology.

In the last annual report of Limagrain, our chairman of the board, farmer in Central France, was summarizing his credo on innovation and biotechnology “The ability to innovate is still the most important factor in the prosperity of companies and people. Our standard of living stems from the discoveries and inventions of previous generations depends on the choice we make today”.

For our know-how, which underlies our activity of crop plants and vegetable breeding, and which relies on decades of experience, what is our need for biotechnology?

Because we want to be and stay at the cutting edge of technology and because we want to make the discoveries and inventions from today and tomorrow support sustainable agriculture, we consider that biotechnologies are the new and irreplaceable tool to improve better and faster plant varieties to fulfill the needs for production, product quality and environment conservation.

Limagrain, a co-op from Central France, and Pau Euralis, a co-op from South West France, have created in 1997 Biogemma, a plant biotech company by merging their former lab activities. Therefore, 17,000 farmers are owners of Biogemma, having the rights to examine our research programs and decide on our research orientations. RAGT, a french SME breeding company joined Biogemma recently.

Limagrain, Euralis and RAGT are not only involved in plant breeding, they are also active in agro-industry: producing feed, canning vegetables, and providing food ingredients they use in part for food production (industrial bread-making). They are at both ends of the chain, deeply involved in agro-food industry.

As a result, their knowledge of the market and its constraints and expectancies is high. They have very early adapted their activities to these characteristics: for example, Limagrain has set up traced corn chains that matches exactly with customer specifications from the creation of new varieties to grain processing.

A typical chart of this chain starts with breeding, integrating agronomic and quality criteria, seed production by a network of farmers, then seeds conditioning and sales for grain production. Following the harvest, the grain is transformed in food ingredients (semolina, pellets, etc).

In a strong commitment, our groups have integrated the progress of biotechnology and genetic engineering to give rise to the creation of better adapted varieties.

As you know it, food products and food ingredients obtained through these new technologies are not considered as traditional food.

In this latter case, traditional food is assumed initially to be safe. It can be marketed immediately, it is not subjected to any regulation and will eventually be submitted to scientific evaluation only if safety problems are arising. Conventional breeding, has given some rare examples of products withdrawn from the market (one potato and some squash varieties).

New technologies have turn this scheme upside down in considering a risk assumption. One has to conduct a preliminary scientific assessment before placing a novel food or a GMO on the market and further assess short- and long-term effects by nutritional and safety post-market surveillance, allowing immediate withdrawal of the product which is the source of the problem.

What are our views on the system?

First, is important to note that this system has been used for the first market approval of GMOs, and we have now more than seven years of experience.

From there, we can make a very clear report: there is no scientific evidence for any adverse effect to human health or the environment of the GMOs so far authorized for marketing. “In 13 years of U.S experience with biotech products, no evidence of food safety risks –not one rash, not one sore throat, not one headache” told Congress David Aaron from the U.S. Commerce Department (reported in The Wall Street Journal). And from the European Commission: “ No peer-reviewed scientific evidence exists for any adverse effect to human health or the environment of the GMOs which have so far been authorised for marketing“

This system, has proved to be efficient, but is totally blocked in the European Union for several reasons. A system based on risk assessment, on a case-by-case basis need to be implemented in Europe, as soon as possible. Scientific evaluation has to be the basis of evaluation, in respect with the European principle of proportionality. Application of this system needs further elaboration of clear tools and references. Among the gaps:

- how to evaluate the potential allergenicity of a protein? For most people, this is a major concern for GM food and food ingredients.
- better define labeling requirements,
- what kind of post-marketing surveillance? This is a powerful tool to accumulate information and to track any potential problem, but has to be precisely defined.
- reference data by species need to be listed.

If the evaluation system is not properly further elaborated, there is an important risk for arbitrary decisions to be taken.

Despite this safe system, public acceptance has never been so low, still with a tendency to become lower and lower. Opponents to biotechnology, if not more numerous, are becoming more radical elsewhere in the world, from Europe where field testing of GM plants is becoming a nightmare for scientists to USA were the number of attacks against research facilities are growing.

If one considers the opinions given on biotechnologies and this system of management of the food safety, and more precisely on plant biotechnologies, this science should be well accepted by the public.

Taking into account the fact that 73% of European consumers rejects GMOs, we are not sure at all that this choice is made in full knowledge of the facts. For example, most of the people are not aware that evaluation and management of food safety is under regulation by European authorities; they don't know that GMOs and Novel food are assessed, prior to their placing on the market, for potential risks, allowing anticipation and management of food safety. From these examples, we think that there is an important need to communicate in order to gain or to restore consumer's confidence.

Furthermore, this communication exercise has to be well conduct and not giving rise to an over assessment or an inadequate assessment. In this case, what would be the foreseeable consequences for European agriculture? For both farmers and consumers, it would be a run towards uniformity of agricultural productions; the cost of deregulation would make valorization of biotechnology products only possible for commodities on large markets. As a consequence, any production's diversification would be impossible, with the lost of potential uses of this technology; the mass production would definitively winning against identity preserved markets which are source of development for quality productions.

Therefore, an important question remains: how is it possible to reconcile food safety assessment and development of quality traits for specific markets? If we are unable to find an answer to this question, in the long term the activity of our co-op groups would be threatened by the impossibility to innovate and diversify.

HARMONISING SUBSTANTIAL EQUIVALENCE – AN INDUSTRY TASK TOWARDS PLANT SPECIFIC CONSENSUS DOCUMENTS

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1 ABSTRACT

Evaluation of substantial equivalence has contributed significantly to the safety assessment of the existing GM crops. It plays an important role by identifying similarities and any differences in the chemical and nutrient composition between the GM crop and its conventional non-GM counterpart. This paper considers in detail the components of the evaluation by describing the framework under which data is collected for the compositional analysis, and the specific constituents for the compositional analysis to demonstrate substantial equivalence in GM maize.

The compositional analysis together with the other important attributes (biological and toxicological analysis) of the safety assessment of GM crops continues to be a useful concept to show that GM crops are “as safe as” conventional non-GM crops.

2 INTRODUCTION

A series of documents on the safety assessment of genetically modified (GM) crops, including the evaluation of substantial equivalence, have been prepared by the Technical Advisory Group (TAG) of EuropaBio’s Plant Biotechnology Unit (PBU). Membership of the TAG consists of regulatory scientists representing the following companies: Advanta, Aventis CropScience, Dow AgroSciences, DSM Group, KWS, Limagrain Agro Genetics, Monsanto, Pioneer Hi-Bred/DuPont, and Syngenta.

The objective of these documents is to establish consensus of the PBU member companies on the data necessary to assess safety of GM crops notified under Directives 90/220/EEC and 2001/18/EC, on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 258/97, concerning novel foods and novel food ingredients. The selection of data is based on the requirements specified in the above legislation, relevant WHO, FAO and OECD guidance documents, and guidance from the EU regulatory authorities. It also incorporates requests for certain additional data by EU Member State Competent Authorities expressed in correspondence to companies with respect to different notifications for the approval of GM crops. Based on these various sources, the documents offer considered rationale of companies in compiling consensus on regulatory data requirements for the safety assessment of GM crops. Final versions of the TAG documents are available at the EuropaBio website (http://www.europabio.org/pages/articles_list.asp?type=4) documents will be regularly revised and reissued with increasing experience on the safety assessment of GM crops.

The evaluation of substantial equivalence has been recognised as an important aspect of the safety assessment of GM crops (OECD, 1993). It has also been recognised that a consistent approach to the establishment of substantial equivalence can be improved through consensus on the appropriate components (OECD, 1997) and work is in progress by the OECD Task Force for the Safety of Novel Foods and Feeds to develop consensus documents on a crop-by-crop basis.

The components of the evaluation have been considered in detail by the TAG, and specific documents describing the data requirements to demonstrate substantial equivalence in maize, oilseed rape, soya bean and sugar beet have been developed. This paper summarises the points described in the TAG documents on substantial equivalence, in particular "Document 1.1: Substantial Equivalence – Maize", thereby reflecting an industry viewpoint on the evaluation of substantial equivalence.

3 SUBSTANTIAL EQUIVALENCE AND SAFETY ASSESSMENT

It is important to realise that substantial equivalence contributes to the safety assessment of a GM crop – it is not a safety assessment *per se.* The safety assessment of the GM crop is based on a multi-disciplinary approach which includes detailed biological and toxicological characterisation comprising information on the recipient and donor organisms, extensive molecular characterisation, protein expression and evaluation, specific toxicity and allergenicity studies, phenotypical and agronomical analyses, feed performance studies, and any additional case-specific studies.

The safety assessment also includes detailed chemical and nutritional characterisation, by determining the composition of the GM crop and comparing it with the composition of the conventional non-GM crop. This is commonly referred to as compositional analysis. The European Commission Recommendation 97/618/EC, concerning scientific aspects and the presentation of information necessary to support notifications under Regulation (EC) No 258/97, states that compositional analysis "should focus especially on the determination of the content of critical nutrients and any critical toxicants and anti-nutritional factors which might be either inherently present or process derived".

Furthermore, the International Food Biotechnology Council (IFBC) report (IFBC, 1990) states that "in evaluating a genetically modified food, a comparison with its traditional counterpart will be necessary in order to determine whether the significant nutrients in the new food as consumed will fall within the range typical of the product. If the new product is found to have essential nutrients in the same range as its traditional counterpart, no further nutritional evaluation of the product would be required". This comparative concept is known as substantial equivalence, and it is embodied in the regulatory policies of many countries such as the US Food and Drug Administration (FDA) policy on GM plant varieties (FDA, 1992). The evaluation primarily identifies the similarities and any differences in the chemical and nutrient (and anti-nutrient) composition between the GM crop and the non-GM crop, with the proviso that significant differences are further investigated by appropriate studies.

4 FRAMEWORK FOR COMPOSITIONAL ANALYSIS

It is important to establish a framework under which data is collected for the compositional analysis.

4.1 Trial numbers and locations

Based on experience, compositional data from a minimum of four locations, consisting of three replicates per treatment, from each of the two growing seasons (total eight trials) would normally be sufficient for the statistically valid assessment.

With regard to trial locations, for notifications under Directives 90/220/EEC and 2001/18/EC for production (cultivation) approval, the data should be collected from trials carried out in the EU and elsewhere, representing a range of agricultural environments which are typical of where the crop is grown. For notifications under Directives 90/220/EEC and 2001/18/EC for import approval only, and for novel food notifications under Regulation (EC) 258/97, data should be collected from a similar number of trials representing a range of agricultural environments which are typical of where the crop is grown, either in the EU or elsewhere.

These requirements can be streamlined if bridging studies show that different locations do not alter the variables selected for the compositional analysis.

4.2 Experimental comparisons

Trials designed to obtain samples for compositional analysis should contain the following experimental treatments for comparison: (a) GM crop, conventionally managed, and (b) non-GM crop (comparable genetic background), conventionally managed. Notwithstanding that compositional data is intended to assess substantial equivalence in the presence of the genetic modification, in the case of herbicide tolerant plants, trials could be designed to obtain samples from herbicide treated plants for analysis, either by inclusion of the following, additional treatment, or substitution of treatment (a) by the following treatment: (c) GM crop, treated with the herbicide to which tolerance has been introduced (only proximates should be analysed, except where additional analysis can be justified, e.g. where treatment (a) is omitted).

In all cases, the comparison of data should be made between the GM and non-GM crop and compared with the range of values given in published literature (e.g. Haytowitz, 1995; Souci *et al*., 1994; USDA, 1993; Notisplus, www; USDA, www). If a range is not available for any particular constituent, an explanation should be given if there is variation of more than 20% from the mean of the non-GM crop (TemaNord, 1998).

4.3 Materials

Composition data should be obtained on grain and/or forage, as applicable, and presented on a dry matter basis.

4.4 Data

The specific data for each GM crop (maize, oilseed rape, soya bean, sugar beet) recommended for compositional analysis to assess substantial equivalence are given in the respective TAG documents (Documents 1.1 to 1.4 on maize, oilseed rape, soya bean and sugar beet respectively; http://www.europabio.org/pages/articles_list.asp?type=4). In cases where the modification of the GM crop is designed to change a specific biochemical pathway, additional variables in the compositional analysis can be included to characterise the effect of the modification.

5 COMPOSITIONAL ANALYSIS FOR GM MAIZE

This paper focuses on the evaluation of substantial equivalence of GM maize and considers the following constituents for the compositional analysis: proximates, carbohydrates, fatty acids, amino acids, anti-nutrients, minerals, vitamins, and secondary metabolites. This selection is developed after detailed consideration of the scientific literature concerning nutritional and toxicants/anti-nutritional factors present in maize.

5.1 Proximates

Traditionally, the analysis of the major constituents of maize, or proximates, has been an effective method to determine the nutritional properties of maize grain from different hybrids. Maize is mainly used to produce animal feeds that are characterised by their digestibility, palatability and energy content. The protein content and quality of the feed prepared from maize is usually not sufficient and often needs to be supplemented with protein-rich fractions derived from additional processing of maize or other crops such as soya bean. Feed formulation takes into account the different nutritional characteristics of the maize grain obtained from different maize hybrids in order to prepare nutritionally balanced rations.

The other major proximates measured in maize grain are carbohydrates, fibre, fat and ash. Moisture and dry matter are also usually measured in order to standardise the values obtained with reference to a known grain moisture content (e.g., at 15%). Analysis of the proximate components is also applicable to the assessment of the substantial equivalence of maize forage used for animal feed.

5.2 Carbohydrates

The greatest proportion of carbohydrate in maize grain consists of starch, comprising most of the soluble carbohydrate present, the remainder being fibre and free sugars. The whole fibre content is measured by the neutral detergent fibre (NDF) method, which gives the approximate sum of cellulose and pentosans (hemicellulose). The amount of cellulose and lignin can be estimated by the acid detergent fibre (ADF) method.

5.3 Fatty acids

Five fatty acids, which account for 90% of total lipid content in maize (Watson, 1982; 1987), are considered to be important for the compositional analysis of maize grain. They comprise the two most common fatty acids, linoleic and oleic acids, and three other fatty acids which are also found at measurable levels: palmitic, stearic and linolenic acids.

There are other fatty acids detected at very low levels (arachidic, behenic, eicosenoic and palmitoleic) but they cumulatively comprise less than 1% of total lipids in maize. The fatty acids that are not reliably detectable in maize are arachidonic, capric, caprylic, eicosadienoic, eicosatrienoic, heptadecanoic, lauric, myristic, myristoleic and pentadecanoic acids.

5.4 Amino acids

The quality of protein produced in maize can be determined by measuring the content of the different amino acids. Eighteen amino acids commonly found in maize (Watson, 1982) are considered to be important for the compositional analysis. They are: alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

With regard to formulation of animal feeds, the most important amino acids are the nutritionally essential methionine and cysteine. Lysine and tryptophan are also important in feed formulation but are present at low concentrations in maize. These two amino acids cannot be produced by non-ruminant animals (such as swine and poultry) or man. Ruminants, however, have micro-organisms in the rumen that can synthesise both lysine and tryptophan.

5.5 Anti-nutrients

Unlike other crops such as potatoes, there are no generally recognised anti-nutrients in maize at levels which are considered harmful (toxic or allergenic) and worthy of quantification or risk management (Watson, 1982, 1987; White and Pollak, 1995). However, certain EU Competent Authorities have asked for the analysis of two anti-nutritional compounds present at higher levels in other plants, trypsin inhibitor and phytic acid. Both compounds are present in extremely low concentrations in maize grain (trypsin inhibitor: 1.9 units/mg dry weight (Del Valle *et al*. 1983); phytate: 0.89% by dry weight (Cheryan, 1980)).

5.6 Minerals

A range of mineral ions are recognised as essential plant nutrients and are directly incorporated into organic compounds synthesised by the plant. Of these, calcium, magnesium, phosphorus, potassium and sodium are required by the plant in significant quantities and, as such, these macro-nutrients are considered appropriate for compositional analysis of maize. Other mineral ions, such as iron, copper, zinc and chlorine, are micro-nutrients which are required by plants only in small quantities, and are incorporated in plants tissues only at trace levels.

5.7 Vitamins

Maize is not considered an important dietary source of vitamins for either humans or animals. The contribution of maize-based food ingredients to the Recommended Daily Intake (RDI) for humans is calculated to be in the range of only 0.2-1.7% for a typical daily intake of 14g of maize flour and/or meal. For this and other compounding factors, maize meal and flour are usually enriched with wheat flour and other nutrients to provide a more balanced food for human consumption. Similarly, in modern feed formulation, nutritional balance is achieved by admixture of vitamin supplements (Watson, 1987).

Nonetheless, four vitamins (B1, B2, E and folic acid) have been identified for which maize makes a minor contribution to the diet and which are considered appropriate for compositional analysis. In considering the major carotenoids and tocopherols, only β-carotene (provitamin A) and α-tocopherol (vitamin E) are identified as of potential nutritional importance (Watson, 1987). However, the inherent instability of carotenoids necessitates the admixture of vitamin A to feedstuffs. Moreover, levels of carotenoids and tocopherols in maize can vary substantially according to the maize hybrid.

5.8 Secondary metabolites

Secondary plant metabolites are defined in the literature as those natural products which do not function directly in the primary biochemical activities which support the growth, development, and reproduction of the organism in which they occur (Conn, 1981). Only where they are nutritionally significant toxicants (e.g., solanine in potatoes, glucosinolates in oilseed rape) have components from many of these classes of secondary plant metabolites been previously examined on a routine basis as part of the compositional analysis.

However, for the purposes of assessment of substantial equivalence under Regulation (EC) No 258/97, the Competent Authority of the Netherlands has asked for analysis of certain secondary metabolites in maize; those specified are coumaric acid, ferulic acid, inositol and raffinose, for which the range of concentrations in maize is known, and furfural. It is recognised that, as *rapporteur* for submissions made under Regulation (EC) No 258/97, the Competent Authority of the Netherlands requires analytical data on these secondary metabolites

6 AGRONOMIC VARIABLES

Subject to the specific purpose of the genetic modification, certain agronomic variables based on the plant phenotype are recognised as primary indicators of orderly crop growth and development and have, therefore, been selected for the overall assessment of substantial equivalence of a GM crop. The agronomic variables for GM maize can include: plant count at full emergence (e.g., growth stage V3) and/or at harvest; time to flowering (silk emergence and/or pollen shed); appearance (e.g., vigour/colour/leaf rolling); susceptibility to pests and diseases; and, yield at known moisture content. The experimental control would normally be a non-GM maize of comparable genetic background. It should be noted that agronomic variables are highly influenced by the environment.

7 SPECIFIC REQUIREMENTS FOR GM TRAITS COMBINED BY TRADITIONAL BREEDING

Member States have interpreted the scope of Directive 90/220/EEC and Regulation (EC) No 258/97 to require additional notifications for plants in which two or more genes, originally introduced by separate transformation events, have been combined (stacked) in a single plant by traditional plant breeding methods.

In response to the EU notifications, compositional and phenotypic analysis for these stacked plants would be undertaken over a single growing season (at 4 locations), and comparisons made either with the single-event GM plants or with the non-GM control of comparable genetic background.

8 CONCLUSION

Evaluation of substantial equivalence has contributed significantly to the safety assessment of the existing GM crops. It plays an important role by identifying similarities and any differences in the chemical and nutrient composition between the GM crop and its conventional non-GM counterpart. The compositional analysis together with the other important attributes (biological and toxicological analysis) of the safety assessment of GM crops continues to be a useful concept to show that GM crops are “as safe as” conventional non-GM crops.

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SAFETY ASSESSMENT OF GM-FOODS: THE SUBSTANTIAL EQUIVALENCE AND ENVIRONMENTAL INFLUENCES

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1 SUMMARY

In the safety assessment of genetically modified foods the concept of the Substantial Equivalence is still important to direct and instruct the risk assessment. However, it must be clear that the Substantial Equivalence is not a safety assessment per se. It is rather a starting point for a comparison between the genetically modified organism and its closest traditional counterpart and can help to identify intended and unintended differences on which further safety assessment should be focused. As problems of unintended effects are not unique for foods derived by genetic modification the same risk assessment schedules should apply also to products obtained with other modern techniques for food production.

Environmental factors can influence conventional and recombinant gene products in food. Effects of such factors may be regionally highly different due to environmental conditions. Multiple environmental signals such as light, temperature, stress factors or pests have been shown to activate gene expression or to modify gene products. Recently, the importance of environmental impacts on gene expression resulted in a discussion on a new view of a flexible, or fluid genome which corresponds tightly with the environment for an appropriate gene expression.

The risk assessment of genetically modified plants or foods therefore needs to address such influences by applying non-targeted or targeted approaches. For non-targeted approaches modern molecular profiling techniques such as comparing gene expression by microarray techniques, substractive hybridisation to detect environmentally activated recombinant genes, or proteomics for the analysis of potentially new protein products derived by unexpected mechanisms such as post transcriptional modifications will soon be available.

2 CONCEPT OF SUBSTANTIAL EQUIVALENCE AND RISK ASSESSMENT OF GM-FOODS

It is now generally accepted that the concept of the Substantial Equivalence is not a safety assessment. It is a starting point for a safety assessment by comparing the genetically modified organism and its closest traditional counterpart. This is the basis for an identification of intended and unintended differences on which further safety assessment should be focused. Based on this starting points the risk assessment especially needs to assess especially the genetic modification, nutritional properties of potentially newly expressed substances, unintended effects, food consumption patterns and the influence of food processing.

3 ANALYSIS OF UNINTENDED EFFECTS

The reasons for unintended effects can be diverse, e.g. a random integration of transgenes, insertional mutagenesis, disruption of endogenous gene functions, gene activation/inactivation, production of new proteins or changes in enzymes, metabolites or the phenotype. However, it must be emphasized that unintended effects have also been reported in products derived from conventional breeding. The safety assessment of unintended effects in transgenic foods can follow targeted approaches such as specific analysis where effects are supposed. Alternatively, non-targeted approaches have to bee applied: Modern molecular methods have been developed recently, which can be used for the analysis of complex gene and protein expression. Especially profiling techniques such as comparing gene expression by microarray-techniques, substractive hybridisation to detect activated recombinant genes, or proteomics for the analysis of potentially new protein products derived by unexpected mechanisms such as post transcriptional modifications will soon be available for a routineous use.

4 ENVIRONMENTAL INFLUENCES ON GENE-EXPRESSION IN GENETICALLY MODIFIED ORGANISMS

Multiple environmental influences have been shown to activate or modify gene expression in microorganisms, plants animals or man. Molecular routes of signalling to the transcriptional or posttranscriptional machinery of cells have been investigated for signals such as light, temperature, stress factors or pests. Especially for different temperature conditions specific effects has been shown to effect alternative splicing, mRNA processing and accumulation of transcripts e.g.for the granule-bound starch synthase in rice (Larkin 1999).

A comparison of specific crop- compounds, antinutrients, in GMO-plants and their conventional counterparts has shown no unexpected differences. However, in both, GMO-plants and conventional plants environmental conditions such as drought have significantly changed antinutrient levels, such as glucosinolates in rape (Nowak, Haslberger, 2000, Fig 1). In general, in this study it became evident that the risk assessment of genetically modified foods is seriously hampered by a lack of important data in dossiers of GM-products and by missing data or experience on standard concentrations and tolerable variations of plant ingredients.

For a risk assessment of potential environmental influences on gene expression or gene products of GMO-organisms, the environmental effects on gene-expression in conventional organisms needs to be compared with effects of genetically modified organisms. Especially for such a comparison molecular profiling techniques are adequate.

The importance of the analysis of environmental factors on gene expression in GMO-plants and GMO-foods reflects a change of the view on gene-expression and the genome. The importance of environmental influences on the function of genes in all area of life sciences is getting generally accepted and points to the direction of a flexible or fluid genome which interacts with the environment for appropriate protein responses (fig. 2).

5 RESEARCH NEEDED

The consequences of environmental impacts on organisms, and genetically modified organisms has prompted specific questions in this area. Field experience seem to be necessary for a better understanding of the importance of environmental influences as well as for improved methods for a risk assessment of genetically modified organisms. Important questions that need to be addressed are specifically:

- the impact of environmental variability on crops
- the establishment of a profile of gene-expression
- protein accumulation during crop development under the defined environmental conditions
- identification of mechanisms of gene regulation that coordinate the response of the crop to specific environmental cues.

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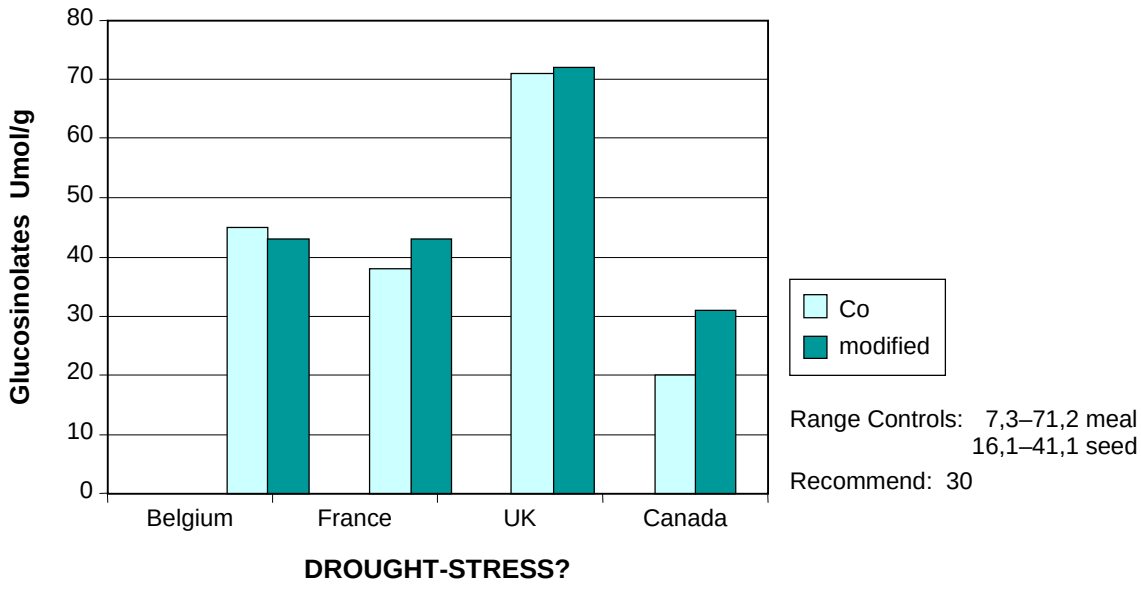

Figure 1: Effects on environmental factors on Glucosinolates in PGS- Rape

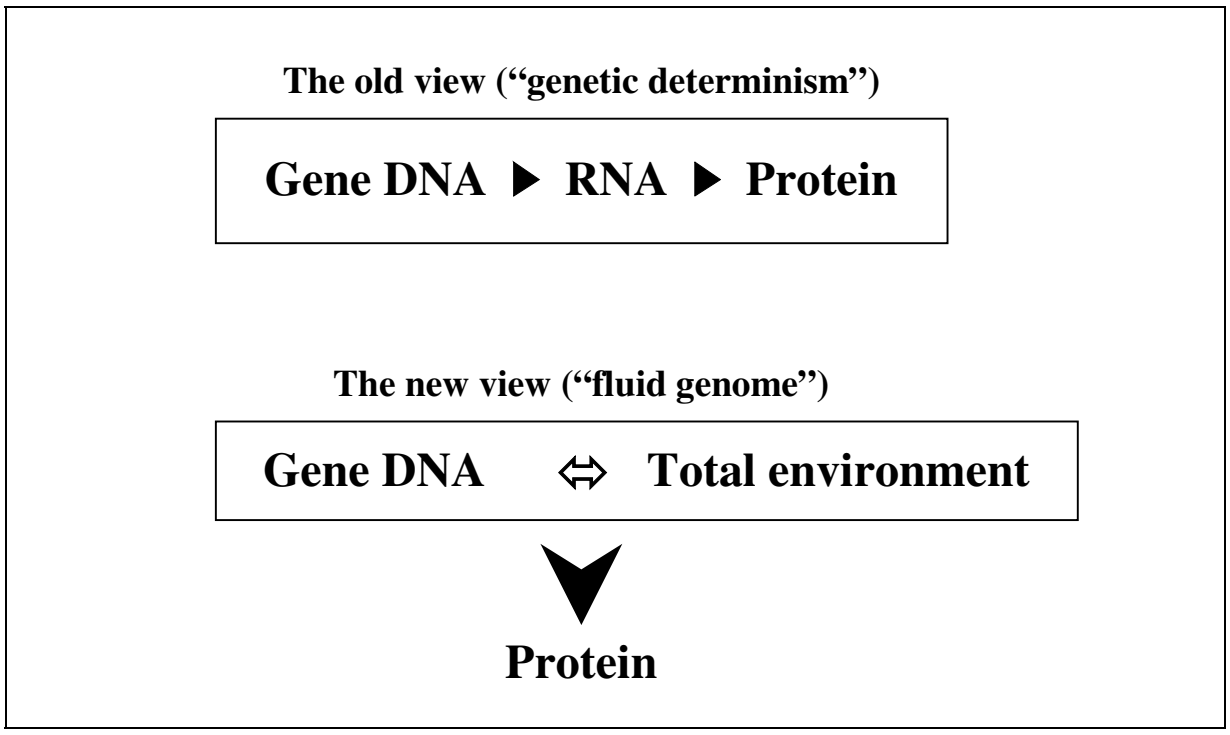

Figure 2: Environment and gene expression

GM FOOD CROPS AND SUBSTANTIAL EQUIVALENCE IN THE EU

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1 ABSTRACT

The concept of substantial equivalence plays a pivotal role in the safety evaluation of the food use of (ingredients of) GM crops. In the European Union, under the so-called Novel Foods Regulation (258/97), application of this concept also triggers whether a 'light' notification or a 'heavy' authorisation procedure has to be followed. Against this background, several notification dossiers for products derived from GM varieties of maize and refined oil derived from GM varieties of oilseed rape have been analysed. The analysis showed that compositional data submitted on the content of macro- and micronutrients, vitamins, inherent plant toxins, and anti-nutrients lacked consistency from case to case. Furthermore, the design of the GM crop field trial, the geographical locations and seasons of planting and harvesting, and the choice of control differed considerably from case tot case.

2 INTRODUCTION

In the EU Regulation 258/97 on Novel Foods and Novel Ingredients regulates the food use of (ingredients of) genetically modified (GM) plants.[1] 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.

Against this background the Dutch Foundation 'Consument en Biotechnologie' was 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 was 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 has been 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

[1] 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.

of national competent authorities and the European Commission. In addition, BEUC, participating in ENTRANSFOOD[2], a European research project on the food safety assessment of genetically modified food crops, has been enabled to provide adequate input into this research project. Within this context the Dutch Foundation 'Consument en Biotechnologie' commissioned Schenkelaars Biotechnology Consultancy[3] 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.

3 MAIN FINDINGS

3.1 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.[4] This assumption has however been challenged in scientific literature and empirical data are lacking to validate the simple linear model of 'precise' genetic engineering.[5, 6]

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.[7] 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. [8, 9] These meetings resulted in en-

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[2] 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 athttp://www.entransfood.org).

[3] GM food crops and substantial equivalence in the EU, Schenkelaars Biotechnology Consultancy, in commission of the Dutch Foundantion 'Consument & Biotechnologie, July 2001 (See http://www.sbcbiotech.nl).

[4] OECD, Recombinant DNA Safety Considerations, 1986.

[5] 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.

[6] 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).

[7] 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.

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

dorsement 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.

3.2 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.[10]

When Regulation 258/97 came into force in January 1997, an operational definition of the concept of substantial equivalence was not available.[11, 12] 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. [13]

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.[14]

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

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

[11] 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.

[12] 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.

[13] 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.

[14] 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.

3.3 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.[15]

3.4 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.

3.5 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

[15] See footnote 3.

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.

3.6 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.

4 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.

SAFETY OF CONVENTIONAL CROPS AS A BASIC ASSUMPTION IN SUBSTANTIAL EQUIVALENCE

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1 ABSTRACT

Conventional plants as comparators to genetically modified plants are not necessarily as safe as they should be. The argument, that conventional plants has been used safely for centuries, is not a valid one. The concept of substantial equivalence should not be used, as one of the foundations, i.e. the safe counterpart, is missing.

2 MAIN FINDINGS

The evaluation of the toxicological part of 9 applications for authorisation of the release of genetically modified plants under the EU directive 90/220/EEC, performed in cooperation with the Federal Environment Agency (UBA) and the Inter-University Research Center for Technology, Work and Culture (IFZ), revealed some remarkable results:

No experimental toxicological investigation was performed with the whole plant or with plant products. Instead of, it was tried to show that the insert is safe and that the concept of substantial equivalence could be applied. The concept of substantial equivalence was applied in 7 out of 9 cases.

It was interesting to note that a lot of doubtfull assumption based reasonings were found in the dossiers – and in parts also in the reports of the authorities. Some of these assumption based reasonings may be true, but are not proven or validated, some other are not reliable at all. This was interesting because – to my impression – the risk assessment of the genetically modified plants was to a much greater extent an assumption based process compared to risk assessments of chemicals, pesticides or drugs.

Two examples of assumption based reasonings, which were found in the dossiers and which are not justified, are the following:

- "It is well established that the gene product XY has been safely consumed throughout humankind's existence, and is not associated with any health concerns."
- "The conventional counterpart (of the GM plant) is used for centuries and is (therefore recognised as) safe".

The 2 arguments look reasonable at first glance, but they are not, as I will explain in a minute.

Assumption based arguments are not only found in the dossiers of the applicants, but also in fundamental papers on substantial equivalence and in the discussion of it. For example in the description of the principle of substantial equivalence in the original OECD paper of 1993 (3):

"Historically, foods prepared and used in traditional ways have been considered to be safe on the basis of long-term experience, even though they may have contained natural toxicants or anti-nutritional substances. In principle, food has been presumed to be safe unless a significant hazard was identified."

Some doubt is contained in these sentences, as to wether the conventional food is really safe, but nevertheless the concept of substantial equivalence is based on the presumable low risk of it.

I would like to concentrate in my talk on the discussion of the unproven assumption, that the conventional plants are safe, because they have been used – safely? – for centuries.

2.1 Many varieties over the years

The nowadays used crop lines were developped over the years and are probably in most cases not any more equivalent to those lines used some decades or some centuries ago. Crops could have quite another toxicological characteristics today than centuries ago. It should be remembered that traditional breeding even produced a few - reported - varieties, that were obviously toxic. Well known is the celery, that was not only resistent against insects but also produced a high concentration of psoralen, a carcinogen and phototoxin.

Therefore, the reference to "the" crop, that has been used for centuries, is misleading and not justified.

2.2 Are crops, that are used as food, really safe?

Doll and Peto (2) reported 20 years ago an epidemiological investigation, that showed that about 35 % of the spontaneous cancers of humans are produced by the diet. Although the figure of 35 % is by no means an exact one, it should serve here to raise or strengthen the suspicion, that food might not be as safe as it should be, but that it likely contributes to the cancer rate.

Some plants could be better off than the diet in general, as it is known, that a higher consumption of vegetables and fruits diminishes the cancer rate in humans. It is not expected that this reduction of the cancer rate is valid for each of the vegetables and fruits and also not for each of the plants in general.

2.3 Which hazards of crops can be detected by the public, without applying scientific methods?

The individual, the family doctor or even the pathologist can detect – by simple means – a causual relation between an unknown toxic agent and the toxic action in only a few situations:

They can detect the causual relationship especially if the time between the the ingestion or exposure and the lesion is short. For example the acute toxic action after the ingestion of deadly nightshade (belladonna) was probably recognized already long time ago without much scientific effort.

Also some chronic toxic effects, that manifest only after a long latency period or after chronic exposure, can be recognised, but only if the effects are spectacular or massive. For example mesothelioma was correlated with the inhalation of asbestos, although the cancer manifested only years to some 10 years after exposure. This was possible, because mesothelioma is a cancer only very rarely seen before and thereby the first cases caused alertness in the medical profession and the causation was soon established.

If asbestos inhalation would have caused "only" an increase of the incidence of common lung cancer instead of mesothelioma the relationship to asbestos would not have been revealed, especially as the long time between exposure and toxic action will obscure it.

That is to say, that effects like chronic toxicity, carcinogenicity or reproduction toxicity can not be associated with the causual agent unless the effect is very massive or spectacular. More subtle effects might be overlooked even after many years of usage of the agent.

Statements like
"This food has been used commercially for 4 years, and 300 million Americans are currently eating it with no sign of a problem"
(written by a former chairman of one of the advisory committees, in Nature 1999) are therefore by no means a prove for a low risk of this particular food, but only an indication that easy to observe short term effects are missing and that very massive or spectacular long term effects are not – yet – encountered.

If an old food, used over centuries, is considered, not even spectacular or massive toxic effects would be detected nowadays, because these effects would have occurred centuries ago, when the food was newly introduced, and nowadays the effects are not any more spectacular but possibly form a part of the background of diseases seen in humans.

2.4 Which methods can detect more subtle toxic effects?

Toxic lesions, including more subtle lesions, which occur delayed or after long term exposure, can be detected in standardised toxicological experiments, mainly animal experiments.

Epidemiological studies may also serve this purpose in some cases, especially if there is already a suspicion.

2.5 Have the conventional crops been subjected to systematically toxicological evaluation, so that they can claim to be safe?

Are long term experiments or epidemiological studies available for the usual crops?

A literature search for maize, corn, Zea mays and rape, rapeseed, Brasssica napus and carcinogenicity, cancer, chronic toxicity etc. in the toxicological and nutritional databases produced a lot of reports on contaminations, on single ingredients of the crops, etc. but no carcinogenicity or long term toxicity study with the plant. An exception are studies with corn oil. But these studies were not performed because corn oil is a food but because corn oil is used as a vehicle to to dissolve chemicals, pharmaceuticals and so on in toxicity studies.

The generalisation, that conventional crops are not investigated toxicologically in a standardised way, from 2 crops to each of the crops, is endorsed by the recommendations to the Novel Food Directive of 1997 (1):

> *"Foods are usually complex mixtures of macro- and microconstituents which provide energy and nutrients and contribute to the well-being of humans. They have traditionally been regarded as natural, beneficial and necessary products whose safety and nutritional value need not be questioned. Regulatory approaches to food safety have reflected this attitude and have focused on food additives, processing aids and contaminants of natural or industrial origin.*
>
> *Thus, foods have not hitherto been systematically subjected to nutritional or toxicological evaluation, except in rare cases where acute toxic effects have been reported in humans (e.g. solanine, cyanogenic glycosides) or in those cases where animal studies or human experiences have suggested adverse effects from raw food materials (e.g. raw soya flour). ... On the other hand, food additives are not permitted in food unless they have been subjected to exhaustive toxicological evaluation."*

Isn't it strange that food additives or even chemical intermediates, which are released only in case of an accident, are subjected to a systematically toxicological evaluation, wheras food constituent, which are consumed in large amounts, are not?

3 SUMMARY AND PROPOSED CONSEQUENCES

The crops can not be considered a priori as safe, only because they or their ancestor plants have been used for long times without producing obvious damages to the health or the environment.

It is realized, that the implementation of the following proposals is not easy task and requires the development of scientific and administrative methods.

- Conventional plants are not a reliable foundation for the application of substantial equivalence to GM plants, because they have not undergone a systematical risk assessment.

  A new plant line should not only have a better performance in an agronomic sense, but also have a lower risk than the old plant line.

  For comparison: The same requirements are clearly found at the introduction of a new medicine.

  The concept of substantial equivalence should at most be applied, if the conventional counterpart is already toxicologically investigated.
- The risk assessment of GM crops should be based on toxicological results and on the estimated exposure.

  Seeds of GM crops are marketed by only a few companies, in great volumes. It is therefore possible, and to my opinion also justified, to require the toxicological testing of the whole plants or their products, before authorising the release of the GM crops.
- The methods of toxicological testing of GM crops should be improved and adapted to the specific needs of foods and their constituents.
- The testing of GM crops could be a starting point to the systematical toxicological investigation of conventional crops.

  A similar regulatory process was started about 20 years ago with new and existing chemicals. First the new chemicals were subjected to a risk assessment, the existing chemicals are assessed now, step by step.

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AN ASSESSMENT OF THE PRINCIPLE OF SUBSTANTIAL EQUIVALENCE REGARDING THE EVALUATION OF ALLERGENIC EFFECTS OF GENETICALLY MODIFIED ORGANISMS

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1 ABSTRACT

The principle of substantial equivalence is suggested for the evaluation of the allergenic potential of foreign proteins introduced in genetically modified organisms (GMO). It is based on the assumption that proteins which are either very similar or identical to other known proteins will exhibit comparable characteristics. Several examples from the recently published literature in the field of allergology demonstrate that this principle can neither be applied to predict the allergenic potential of a given protein nor to that of a complex GMO containing this protein.

2 INTRODUCTION

The risks and benefits of genetically modified organisms (GMO) have to be assessed to ensure consumer safety and human health. The review of eight applications for registration of GMO in the European Union showed that none of the 8 applications contained any experiments studying the allergenic potential of respective GMO. The applicants mainly made attempts to downplay the allergenic risk of the GMO utilizing the principle of substantial equivalence. Several of the arguments based on the principle of substantial equivalence were found to be incorrect.

3 HIGHLY HOMOLOGOUS PROTEINS HAVE SIMILAR ALLERGENIC POTENTIAL

Several recent studies showed that it cannot be concluded on the basis of sequence comparisons that a particular protein will or will not exhibit allergenic activity. This is demonstrated by the fact that mutants which differ from the major birch pollen allergen Bet v 1, by only a few amino acids showed almost no allergenic activity (1). These mutants were generated to reduce the allergenic potential of the Bet v 1 protein to convert it into a safe immunogen for immunotherapy. It has also been demonstrated that birch pollen contains naturally occuring isoforms of the Bet v 1 protein presumably with similar biological functions. Although they differ from the highly allergenic Bet v 1a isoform only in a few amino acids, their allergenic potential is greatly reduced (2, 3).

4 THE PROTEIN EXPRESSED IN THE CONTEXT OF THE COMPLETE GMO BEHAVES IN THE SAME WAY AS THE ISOLATED PROTEIN

In our opinion it is insufficient to study the allergenic potential of isolated proteins because their integration into a complex organisms may lead to pleiotropic effects which may cause altered expression of other highly allergenic components. That factors, which *per se* are not related to allergy can have a dramatic influence on the expression of allergens is illustrated by two examples. Certain allergens are preferentially expressed in certain tissues (e.g., pollen) but to a lesser extent in somatic tissues. Moreover, pollen maturation leads to rapid and strong upregulation of allergen expression (4). Additionally, it has been shown that elevated levels of ozone increase the allergen contents in plant pollen (Hayek B. and Valenta R., unpublished data). We therefore believe that it is necessary to compare the allergenic potential of the complete GMO with that of the wildtype organism rather than studying the introduced component as isolated substance.

5 PROPOSED ALTERNATIVES TO THE PRINCIPLE OF SUBSTANTIAL EQUIVALENCE

There are at least two possibilities to evaluate the allergenic potential of a complex GMO. First, it is possible to screen GMO and wildtype extracts with allergen-specific probes to search for expression of allergens (5). Second, the sensitization capacity of a GMO can be evaluated by immunization experiments which compare GMO and wildtype extracts for their capacity to induce IgE antibodies, the carriers of allergenic activity (6).

In conclusion, the application of the principle of substantial equivalence alone is insufficient to estimate the allergenic potential of GMO. Simple experiments can be performed to investigate the allergenic potential of GMO.

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DEALING WITH UNINTENDED EFFECTS

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1 SUMMARY

Safety assessment of genetically modified foods is based on the concept of *Substantial Equivalence*, which implies the identification of possible similarities and differences between a genetically modified food and its traditionally produced counterpart, which is considered to be safe for human consumption. A systematic comparison is made of the agronomical, morphological and compositional characteristics. Identified differences will be further investigated with respect to human and animal health, which may include detailed (immuno)toxicological and biochemical testing. One of the hazards which should be identified and further characterised is the potential occurrence of the so-called unintended effects due to the genetic modification process. Normally chemical analysis of single known macro and micro nutrients and other specific compounds in the modified and parent organism is performed. In order to further improve the chances to identify unintended effects, new profiling methods are under development. Such methods allow for the screening of potential changes in the modified host organism at different integration levels, i.e. at the genome, the gene expression and protein translation level, and at the level of cellular metabolism. Principles and limitations of such methods will be described, which are of particular interest for genetically modified organisms with multiple gene insertions. The concept of *Substantial Equivalence* is an important part of the safety assessment strategy for genetically modified organisms. There is a need for further standardisation and uniform application of the concept.

2 CONCEPT OF SUBSTANTIAL EQUIVALENCE

The concept of *Substantial Equivalence* as a guiding tool for the assessment of genetically modified foods has been formulated by the OECD (1993, 1996, 1998), and further elaborated by FAO/WHO (2000).The concept is a *comparative* safety assessment strategy based on the idea that existing traditionally produced foods can serve as a reference, since they are considered to be safe through a long history of use. Application of the concept provides insight in the occurrence of potential differences in the agronomical, morphological and compositional properties between the genetically modified organism and its conventional counterpart, but does not assess these differences. Establishment of Substantial Equivalence is only *part* of the safety assessment procedures which is further build up of:

- Molecular characterisation of the genetic modification process
- Toxicological assessment of newly expressed proteins
- Identification and assessment of the potential occurrence of unintended effects as result of the genetic modification and its impact on human health
- Evaluation of the potential for gene transfer from genetically modified foods to human/animal gut flora
- Assessment of the allergenic potential of the newly inserted trait(s), and of the whole modified food
- Evaluation of the role of the new food in the diet.

2.1 Is the Concept of Substantial Equivalence based on valid scientific principles?

The traditional food supply is taken as the basis for a comparative safety assessment of genetically modified foods. It should be pointed out that safety assurance of our existing foods is primarily based on long-term experience and history of use, which learned that even though foods may contain anti-nutritional or toxic substances, they are safe, nutritious and healthy. Safety testing of whole foods with respect to chronic consumption has not been carried out systematically. Centuries of careful selection and classical breeding has yielded a safe and wholesomeness food supply. Knowledge on the relation between diet components and human health is growing rapidly with the introduction of new molecular techniques, and therefore a more targeted plant breeding strategy may provide even more healthier and safer foods.

Selection of key compounds for comparison is essential for the establishment of Substantial Equivalence

Key macro- and micro-nutrients, anti-nutrients and plant specific toxins should be included in the comparative analysis of genetically modified varieties with their traditional counterpart, which fulfil an essential role with respect to the nutritional and safety impact of consumption of the food. Furthermore analyses of compounds which are important intermediates of metabolic pathways may reveal differences between the modified and the non-modified organism, which are predictable of expected and unexpected effects which may take place as result of the genetic modification.

Difficulties in the application of the concept of Substantial Equivalence are:

- Determination of genotypic versus phenotypic variations in food crop components
- Standardised performance of field trials
- Standardised statistical analysis of compositional data
- Determination of natural background variations (quality of data, applied analytical methods).

It is therefore of great importance that harmonisation and standardisation is reached with respect to application of the concept of *Substantial Equivalence*, i.e. selection of critical compounds, performance of field trials, establishment of the natural baseline characteristics. Consensus Documents have been formulated by OECD for the establishment of compositional characteristics of a number of food plants like soybean and rape seed, while documents for corn, potato, sugar beet, and rice are in progress (OECD, 2000).

Depending upon the results of the comparison of the properties of the new food with its traditionally grown product, further extensive toxicological testing of newly expressed proteins and of compositional changes possibly occurring as result of the genetic modification is needed. Testing of whole foods, although difficult to perform, is generally recommended in cases of foods which exhibit extensive genetic modifications, which do not possess a history of safe use, and which comprise an essential part of the diet (WHO/FAO, 2000). Thus the safety assessment of genetically modified foods comprises more than just a chemical analysis of the composition.

2.2 Detection and characterisation of unintended effects

Identification and assessment of the occurrence of unintended effects in genetically modified organisms due to the genetic modification process is an essential part of the safety assessment. It should be pointed out that the occurrence of unintended effects is not unique for the application of modern recombinant techniques, but occurs also frequently in conventional breeding. Effects can be predicted on the basis of information on the place of insertion in the

DNA of the host organism and function of the inserted trait or its involvement in metabolic pathways (*predictable*), while other effects are *unpredictable*, due to a lack of information on gene regulation and gene-gene interactions.

Approaches to detect (un)intended effects on the physiology/metabolism of modified organisms are (i) chemical analysis of single known nutrients and toxicants (*targeted approach*), and (ii) profiling/fingerprinting at different cellular integration levels (*non-targeted approach*).

Single compound analysis

Expected changes in the metabolism as a possible result of the genetic modification, can be identified by analysis of a number of specific components based on knowledge of the function of the expressed gene products, while unexpected changes may be identified by chance. Therefore the targeted approach has limitations with respect to detection of unknown anti-nutrients and natural toxins, and is further limited by the availability of adequate detection methods.

Profiling (non targeted) approach

The use of profiling techniques allow for the screening of potential changes in the physiology of the genetically modified host organism at different cellular integration levels: at the genome level, at the gene expression and protein translation level, and at the level of expression of primary and secondary metabolites. These methods comprise DNA analysis, DNA/mRNA micro-array hybridisation, proteomics and chemical fingerprinting (Kuiper et al., 2001).

Profiling methods, although promising for identification and characterization of unintended effects, needs further development and are still not suitable for routine analysis. Standardisation of sample preparation, validation of measurements and treatment and interpretation of large sets of data is focus of ongoing research within an EU-funded project, GMOCARE (www.rikilt.wageningen-ur.nl/euprojects/gmocare.html). The use of profiling methods is of particular interest for those organisms with complex multiple gene insertions, in which the likelihood of occurrence of unintended effects may be increased.

3 CONCLUSIONS

The concept of Substantial Equivalence is an important tool to identify safety issues related to genetically modified products. It is not a safety assessment procedure per se. Depending upon the results of the comparison specific toxicity testing of genetically modified products may be required. Further refinements in methodology for the detection of unintended effects are needed. Screening for potential changes in the properties of genetically modified organisms as result of the modification process becomes more important for those organisms with complex genetic alterations. Application of the concept of *Substantial Equivalence* needs further harmonisation with respect to selection of critical compounds, performance of field trials and statistical analysis.

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SUBSTANTIAL EQUIVALENCE IN VARIABILITY, LESSONS FROM TRADITIONAL BREEDING

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1 SUMMARY

Breeding using traditional as well as contemporary methods entirely depends on variability. Breeders often employ methods to increase variability for the purpose of varietal selection. Crossing and somatic hybridization are principal means of genetic recombination to produce a diversity of genotypes and phenotypes. A large repertoire of techniques is available and can be applied to various kinds of genetic resources that are characterized by their breeding system and genomic compositions. Variability resulting from using these techniques also covers a wide range, and in many cases, variability cannot be used as an indicator of a specific breeding method applied. However, a few breeding methods are characterized, and limited to specific objectives, by the limited variability they employ. Transgenic approaches could complement traditional approaches to increasing, and to reducing, variability. From limited evidence available to date it would seem complicated to distinguish traditional from transgenic breeding approaches by the inherent variability. However, specific features of variation could be used to deduce the technical perfection of specific transgenic experiments. Changing the composition and contents of potato steroidal glycoalkaloids that are potential food toxicants, through breeding and transgene techniques is examined.

2 INTRODUCTION

The concept of substantial equivalence has been adopted by the World Health Organisation as a decision threshold standard (FAO/WHO 1991). This concept was also included in a 1993 document by the Organisation for Economic Co-operation and Development (OECD), to indicate whether a genetically modified (GM) organism was essentially similar to its traditional counterpart (see Barrett et al. 2001). It was implemented as a decision threshold that "If a new food or food component is found to be substantially equivalent to an existing food or food component, it can be treated in the same manner with respect to safety." To further focus that rather generalizing concept, a Canadian expert panel (Barrett et al. 2001) proposed two interpretations of the principle that constitute

A), a decision threshold interpretation:

A GM organism is "substantially equivalent" if, on the basis of reasoning analogous to that used in the assessment of varieties derived through conventional breeding, it is assumed that no changes have been introduced into the organism other than those directly attributable to the novel gene. If the latter are demonstrated to be harmless, the GM organism is predicted to have no greater adverse impacts upon health or environment than its traditional counterpart.

and

B), a safety standard interpretation:

A GM organism is "substantially 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.

From these definitions it is not immediately clear what the terms "harmless" and "changes" exactly refer to. It is, however, apparent that the principle of substantial equivalence has been introduced with the intention to leave room for interpretation and, at the same time, to further spur the discussion about the potential risks of genetically modified organisms (for an example, see Love 2000). The principle is meant to prevent the release of hazards that could threaten human health or affect components of the environment, in the wide sense. However, often it is not straightforward to determine a specific GM product's inherent characteristics that make it distinct from its traditional counterpart and that might be a hazard or risk. It may even be difficult to find on an objective ground an appropriate traditional counterpart of a GM product. For example, when a clonal crop, such as potato, is genetically modified, the single clonal cultivar of that GM-potato usually is taken as the appropriate counterpart (Love 2000). Frequently it is not taken into account that a clonal cultivar represents a very small part of the range of potato (*Solanum tuberosum*) cultivars, and it is neglected that a breeder, having crossed two clonal varieties, often selects not just one, but several clonal potato cultivars from the cross progeny that may vary widely for characters important to both producers and consumers. Therefore, is it justified to compare a GM-potato to just the single clone that was genetically transformed? Would it not be more appropriate to use a series of clones representing the full range of diverse genotypes that can be obtained by crossing of two parental (clonal) potato cultivars? Love (2000) argued that comparing a genetically modified potato to its non-modified donor variety would be justified because single clonal potato varieties are well-adapted to industrial use and, if such a variety were genetically altered and were sold under the same name as the donor variety, difficulties could arise for both industry and consumers. This argument is probably of small importance, as GM varieties are clearly labeled as such, for both regulatory and commercial purposes.

Moreover, it may be worthwhile to analyse what include "all changes introduced into the organism as a result of the introduction of novel genes". Genetic modification normally comprises a multi-step process, including protoplast, cell, callus, and tissue culture, and other ways of clonal propagation of more or less biologically complete individuals that may result from the very procedures of molecular biology and gene transformation. Frequently, the individual or population carrying the transgene that is to become a new variety, is selected during steps of conventional breeding, from an initial panel of several transgenic individuals. Many, if not all, of these steps and procedures are likely to produce by themselves genetical changes that may influence the ultimate product's performance in a way that makes it distinct from the original donor cultivar. When a GM variety is examined for differences to a "traditional counterpart", is the possibility of introducing changes by these conventional procedures examined as a possible source of these differences? With other words, is the possibility of confounding the possible effects of gene transformation with effects of conventional techniques taken into account when a GM cultivar is examined?

These considerations led us to highlight sources of variation that might be involved in various, frequently applied methods of plant breeding. An attempt is made to characterise different methods of conventional breeding, biotechnology, and genetic transformation by their inherent ranges of variability. It is concluded that the principle of substantial equivalence should be used with caution and a precise description of the procedure that is referred to should be made in every specific case. It also follows that no objective validation of GM plant varieties can be obtained when crop producers and consumers do not have access to these varieties.

3 VARIABILITY OF PLANT VARIETIES, ITS SOURCES AND UTILIZATION

3.1 Variability is used for conventional breeding

Breeding is the process of creating variability and selecting valuable combinations of genes, the total referred to as genotypes. Variability within a plant variety comprises the total of genotypes the variety consists of. There are various ways that lead to increased variation useful for selection by breeders and farmers. The two most widespread kinds of variation that were used by our ancestors are the natural variation within wild populations of a species and the variation upon open pollination that occurs on the plants growing within a farmer's field. Selection from diversity presented in these "natural" ways was carried out for ten millenia and it has led to the establishment of many landraces (for an example, see Quiros et al. 1992). Today, the landraces represent a wide range of diversity and are eagerly sought for and maintained in genebanks, to be used as a valuable source of variability for modern, scientific, breeding (as an example, see Hanelt and Schultze-Motel 1979).

3.2 Breeding systems as causal determinants of variation-generating methods

The traditional and important crop plants include inbreeders as well as facultative outbreeders, obligate, self-sterile outbreeders, and vegetatively propagated clonals. Breeding employs selfing, intraspecific within- and between-varietal crossing, and wide crosses between species to introduce new resistances and other valuable characteristics. The size of variability that is available often depends on a crop's specific breeding system.

3.3 Other methods to increase the variability in plants are used widely

Besides the above indicated, classical and perhaps most "natural" ways of generating variability useful for selection, more recent developments in biotechnology have been employing the natural characteristics of plant genomes and principles of evolution. Mutations were induced and are widely used to increase the commercial value of a single variety grown on a large acreage (as an example, see Neuffer et al. 1968). Mutations comprise changes in the composition and structure of genes, chromosomes, and whole genomes. Methods of mutation breeding include treatment with chemical and physical mutagens, but also cell, callus and tissue culture on selective or non-selective artificial media which favor survival of specific mutations. Often, mutations result from culturing protoplasts, cells, callus, or tissue, without application of any additional treatment. The processes that lead to these mutations are summarised as somaclonal variation (Creissen and Karp 1985; Vasil 1990) although the causal principles may be several and they are poorly understood (Karp 1989; Hamer et al. 2001).

Since several decades, somatic hybridisation has been an accepted breeding method (Ozminkovski and Jourdan 1993; Gerdemann-Knörck et al. 1994; Rokka et al. 1994; Sidorov et al. 1994). Other methods could be mentioned that have been used widely to create more productive varieties in an unorthodox way, such as the grafting of the susceptible, but high-yielding upper organs of one variety onto the rootstock of a resistant variety. Almost all grapevine production and the competitiveness of many fruit crops depend on this technique of grafting. Yet other methods have been developed although their direct contribution to production of food and feed has remained small. These include the use of periclinal and sectorial chimeras (Hirata et al 1990; Noguchi et al. 1992) and artificial infection with attenuated virus strains (Neitzel 1977), among others.

3.4 Sources of variability are genetic and environmental

Variability of a crop variety has a minimum of two components; a heritable, gene-related and an environmental one. Imagine a series of varieties that are produced by different techniques from one donor variety or from a pair of donor varieties. For example, a series of potato varieties could be produced from just one donor by the selection of a spontaneous mutant that occurred in a large field of clonal plants, by the selection after application in the laboratory of mutagenic treatment, after many cycles of tissue culturing, or after genetic transformation. Likewise, several new varieties of a sexually propagated crop, such as barley, could be obtained from two parental varieties upon crossing and selecting for specific characters by traditional plant breeding procedures, or the seed could be subjected to mutagenic treatment, somaclonal variation during tissue culturing, or to genetic transformation. When the series of varieties produced from the same donor(s) are grown side-by-side in a field, and sophisticated methods are applied to minimize environmental variation (as an example, see Anoshenko 1996), the factor environment can be regarded as a constant. Any difference between two varieties from the same donor(s) should then be related to genetic variability that can therefore directly be measured as a relative value (Figure 1).

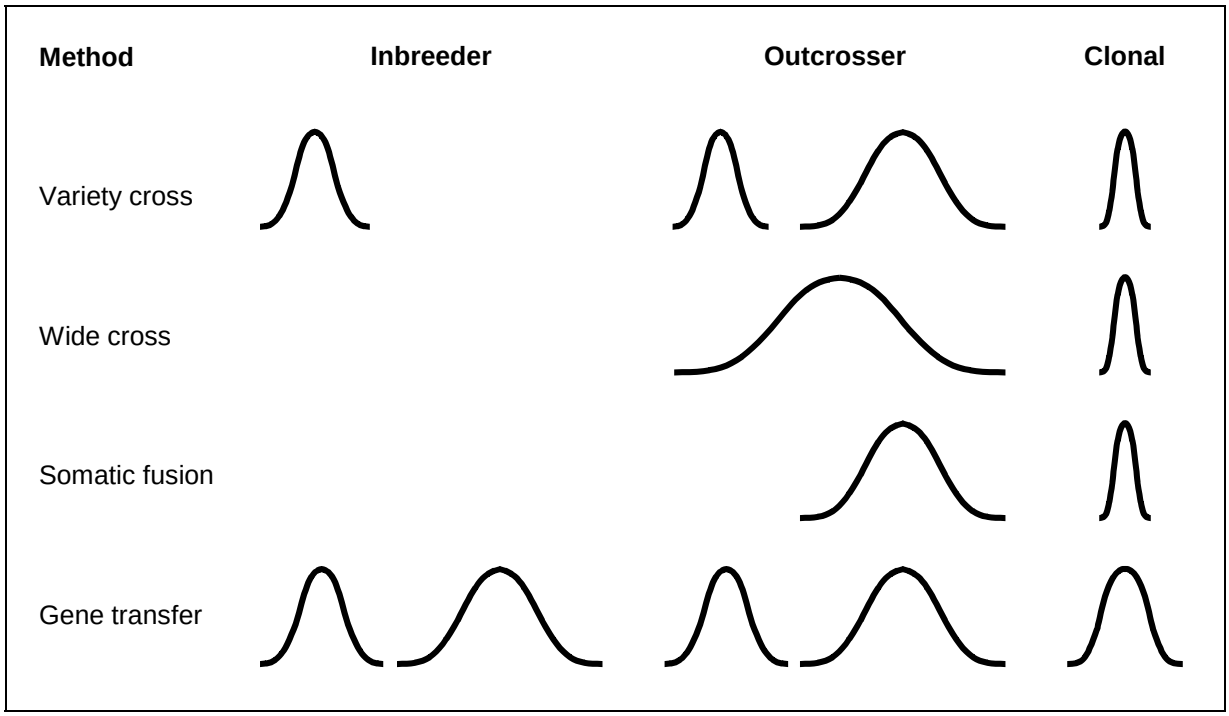

Figure 1: Within-cultivar variability as a function of breeding method. Estimates of relative ranges of variability are indicated by the width of individual normal distribution curves.

3.5 Variability as related to seed production methods

Most commercial varieties demand steady maintenance breeding, otherwise, many cycles of seed production could lead to reduced performance of the variety due to genetic drift and other processes. In general, the methods of seed production largely depend on the breeding system of a crop. Basically, crops propagated by true botanical seed depend on generative reproduction, whereas most clonal crops are multiplied by propagules, such as tubers, storage roots, rhizomes, or sprouts. For each type of propagation and crossing used, a specific, typical scope of variability can be observed (Figure 1).

3.6 The potential impact of genetic transformation on the scope of variability

Most of the classical breeding and biotechnology methods have been depending heavily on random recombination of large parts of the genome. This leads to the need for sophisticated, large-scale selection of the few superior genotypes that are looked for and it often requires large amounts of time and resources. The isolation of a single gene and its transfer to a receptor genotype could circumvent these disadvantages. The number of crossing generations to arrive at a commercially acceptable variety would be fewer. No removal of, and no need to test for the presence of, unnecessary genes accidentally introduced from a crossing partner would be required. A gene introduced via genetic transformation into an existing genotype is expected to segregate as a single factor when it is not interfering with the expression of other genes and when it is not causing any pleiotropic effects. However, the introduction of a single gene also is associated with a number of features that increase the variability of the altered plant above the predicted level. Segregation for unexpected characteristics or quantitative effects are expected to result from:

- A transgene is accidentally inserted into an existing gene and interrupts its expression,
- Positional effects of the transgene,
- The sequence of the transgene resembles the sequence of an original gene, which leads to post-transcriptional silencing as a result of RNA interference.
- Pleiotropic effects, when a transgene alters features of a metabolic pathway in addition to those it was designed for.

Incomplete expression of a transgene or expression in a quantitative manner could result from sub-optimal functioning of its promoter or of the gene itself in the foreign genetic background.

In summary, the various methods of breeding, biotechnology, and genetic transformation to create new varieties possess different specific characteristics that could be summarised as is attempted in Table 1.

Table 1: Characteristics of creating new varieties through general techniques of breeding and biotechnology

Method	Gene pool accessed	Time required	Perfection	Method on target?
Variety cross	narrow	much	perfect	very much
Wide cross	wide	very much	nearly perfect	little
Somatic fusion	even wider	little	nearly perfect	little
Gene Transfer	very wide	very little	imperfect	very much

4 THE CASE OF VARIABILITY OF STEROIDAL GLYCOALKALOIDS (SGAS) OF THE POTATO AND ITS RELATIVES

4.1 Composition, properties, and inheritance of glycoalkaloids

Steroidal glycoalkaloids are secondary metabolites of the Solanaceae. They are a hazard to human health when occurring at high levels (Friedman and McDonald 1997). The common potato produces the major SGAs solanine and chaconine, both possessing solasodine aglycons. They accumulate in the tuber skin and flesh and their total amounts and relative distribution throughout different plant and tuber parts depend on the specific genotype's response to several environmental triggers. The SGA content in tubers depends on age, lighting conditions, wounding, and stage of dormancy or sprouting. The SGA concentration is highest in tuber skin and periderm, but some cultivars also have high tuber flesh-SGA. For example, mechanical injury upon peeling or cutting can result in accumulation of extremely high and dangerous levels of SGAs within a short time, depending on the genetically programmed response of a variety (Friedman and McDonald 1997).

The inheritance of SGAs is quantitative and therefore shows increased variability. Yencho et al. (1998) detected three quantitative trait loci (QTLs) for solasodine and two QTLs for solanidine. Wild potatoes that constitute a valuable genepool for potato enhancement, synthesize many more glycoalkaloids (Deahl et al. 1993; Petersen 1993). Hybrids of potato and its wild relatives can produce novel SGAs that are not seen in any of the parents (Laurila et al. 1996).

4.2 Strategies to reduce SGA contents

As the SGA production is largely controlled by solanidine UDP-glucose glucosyltransferase (SGT) of potato (Moehs et al. 1997), an antisense SGT could be used to reduce the total SGAs formed within a variety to safe amounts. Other genes of the pathway leading to SGA synthesis could also be used to engineer low-SGA potatoes. SGA content was measured in potatoes that carried an invertase gene from yeast (Engel et al. 1997), the transgenic potatoes had reduced SGA contents than non-transformed potatoes of the same variety. In another experiment, sucrose synthase, the potato's native, inherent counterpart to the yeast gene, that plays an important role in the primary starch metabolism, was silenced by antisense technique. Again, a reduction of SGA levels resulted (Gerstner et al. 1999).

These examples demonstrate that the application of genetic transformation technology for the purpose of reducing SGA content could lead to a GM crop that might actually be safer than its traditional counterpart. The transgene used could even be from the same genepool that is also accessible via other methods of recombination (see above). The modification obtained actually constitutes a pleiotropic effect, as genes were introduced with the objective to alter the primary carbohydrate metabolism; the contents of secondary metabolites, SGAs, was changed as an unexpected side effect.

5 CONCLUSIONS

Variability is inherent of, and results from, genetic modification through traditional, conventional, and modern breeding techniques, biotechnology, and molecular biology, and it has at least a genetic and an environmental component. Therefore, variability should be part of the performance evaluation of GM crops.

To match the principle of substantial equivalence concerning potential hazards to human health and the environment, a GM crop should be compared to appropriate traditional counterparts. More than one traditional counterpart may exist and the choice of the specific counterpart may become arbitrary. Therefore, the principle of substantial equivalence is not a tool for rigid and unequivocable decisionmaking.

Different breeding strategies can lead to different degrees of variability. However, there is much overlap.

Variability of transgenic plants can be caused by both direct and indirect (pleiotropic) effects of the transgene. This can occur also upon traditional hybridization.

Alternatively, a transgene approach could be used to adjust the extent of variability.

Problems related to safety of human health and protection of the environment are mainly technological and it is evident that these problems can be resolved at an individual-case base. However, another part, the consumer-related variability of perception and acceptance of GM crops has not been considered here. The consumers opinion may range from realistic, knowledge-based acceptance or rejection of GM crops to anti-Darwinist or even religious rejection of things that appear, or are said to be, non-natural.

It is proposed that the discussion about the pros and cons of the concept of substantial equivalence, although important and interesting, should not delay the implementation of the legislatory framework needed to distribute GM crops. Both crop producers and consumers should have broad access to GM plant varieties to be able to evaluate their inherent properties and to objectively validate these crops.

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In 1998 **Piet SCHENKELAARS** started Schenkelaars Biotech Consultancy, which is commissioned by NGOs, the Dutch government, industry and the EC. In 2001 he prepared, commissioned by the Dutch Consumentenbond and the Foundation “Consument en Biotechnologie“, an analysis of international and European regulatory discussions on the concept of substantial equivalence as input to a workshop convened by the European consumer organisation BEUC and Consumers International. Piet Schenkelaars studied Molecular Biology and Philosophy of Science at Agricultural University Wageningen and worked afterwards for Dutch NGOs, Friends of the Earth Europe, the Ministry of Environment and a communication consultancy.

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Bodo TROGNITZ studied plant breeding and seed production at the Martin Luther University at Halle/ Wittenberg in Germany. He joined ARC Seibersdorf Research, Austria, as a plant geneticist and molecular geneticist in 2000. Before, he worked at the International Potato Center (CIP), Lima, Peru, on the genetics and breeding of the potato and its relatives and Andean root and tuber crops. This work led to the discovery of novel resistance genes, and to the development of genetic maps and markers for disease resistance.

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Workshop

EVALUATING SUBSTANTIAL EQUIVALENCE

October 19th – 20th 2001

Hotel Bellevue, Vienna

Final Programme

Organised by the

Austrian Federal Environment Agency and

Inter-University Research Centre for Technology, Work and Culture (IFF/IFZ)

Contact: workshop-se@ifz.tu-graz.ac.at

AIMS AND PERSPECTIVES

Within the scope of this workshop both the concept of substantial equivalence and the way the concept is applied in risk assessment of genetically modified crops will be critically evaluated. Possible routes of improving or reshaping the concept will be discussed. One focus will be to discuss the usefulness and problems of this concept with respect to potential toxic/allergenic effects of genetically modified plants and foodstuff derived therefrom.

This workshop is funded by the Austrian Federal Ministry of Social Security and Generations and the Austrian Federal Ministry of Economy and Labour.

Contact: workshop-se@ifz.tu-graz.ac.at

FINAL PROGRAMME

1st Session
"Evaluating Substantial Equivalence"

October 19th 2001, 14.00 – ca. 18.15

Chair: Armin Spök, Inter-University Research Center for Technology, Work and Culture, Graz

Introductory Remarks

14.00–14.15 Helmut Gaugitsch, Federal Environment Agency, Vienna

The concept of substantial equivalence – the rise of a decision tool

14.15–14.55 Peter Kearns, Environment and Health Safety Division, OECD

From concept to practise – interpretations of substantial equivalence in the EU and North America

14.55–15.20 Dietmar Pettauer, Scientific Committee on Food, European Commission

15.20–15.45 Brian Ellis, Expert Panel, Royal Society of Canada

Coffee Break: 15.45–16.15

The limitations and potential utility of substantial equivalence

16.15–16.55 Erik Millstone, Science and Technology Policy Research, University of Sussex, UK

Industry viewpoint on evaluation of substantial equivalence

16.55–17.15 Firoz Amijee, Pioneer Hi-Bred International, Brussels

17.15–17.35 Luc Dormoy, Limagrain, Chappes

Open Discussion: 17.35–18:15

Social Event "Heuriger": 19.30

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2st Session
"Improving or Reshaping Substantial Equivalence? – Experiences from Practice and Novel Approaches"

October 20th 2001, 9.00 – ca. 13.30

Chair: Helmut Gaugitsch, Federal Environment Agency, Vienna

Nutritional assessment within the safety evaluation of GM plants and food derived therefrom

9.00–9.30 Ibrahim Elmadfa, Institute for Nutritional Sciences, University of Vienna

The substantial equivalence: risk assessment or concept to evaluate multiple impacts on organisms

9.30–9.45 Alexander Haslberger, Institute of Microbiology and Genetics, University of Vienna

Substantial equivalence – a few case studies in the EU

9.45–10.00 Piet Schenkelaars, Biotechnology Consultancy, Leiden

Safety of conventional crops as a basic assumption in substantial equivalence

10.00–10.15 Heinz Hofer, Austrian Research Centers Seibersdorf

An assessment of the principle for substantial equivalence regarding the evaluation of allergenic effects of genetically modified organisms

10.15–10.30 Birgit Donabauer, Department of Pathophysiology, University of Vienna

Coffee Break: 10.30–10.50

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2nd Session, continued

Dealing with unintended effects

10.50–11.20 Harry A. Kuiper, RIKILT, Wageningen

What constitutes the "traditional counterpart" – lessons from traditional breeding

11.20–11.50 Bodo Trognitz, Austrian Research Centers Seibersdorf

Coffee Break: 11.50–12.10

Panel Discussion: The future of substantial equivalence: next steps to be taken

12.10–13.10 Firoz Amijee, Ibrahim Elmadfa, Peter Kearns, Harry A. Kuiper, Erik Millstone

Concluding Remarks

13.10–13.25 Armin Spök, Inter-University Research Center for Technology, Work and Culture, Graz

End of workshop

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