

**GENETICALLY MODIFIED FOODS AND ALLERGENICITY: SAFETY ASPECTS AND CONSUMER
INFORMATION, WORKSHOP 28-29 MAY 1999**

REPORT

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Executive summary

Introduction

The possible effects of biotechnology on food allergy were discussed within a two-day workshop, workshop *Genetically modified foods and allergenicity: safety aspects and consumer information*. From May 28 to May 29 1999 the Consumer and Biotechnology Foundation in co-operation with EFA (European Federation of Asthma and Allergy Associations) and BEUC (European Association of Consumer Organisations) brought together a group of representatives from organisations such as consumers and patients organisations, industry, retail, government, as well as food toxicologists and allergologists. The outcomes of the workshop is laid down in this document.

Aim

The aim of the workshop was to give a state of the art of the field and discuss a number of issues, such as the values and flaws of the various testing methods, decision-making procedures, the perception of these risks by the public and the need for information for consumers and patients.

Genetically modified food crops

The workshop focussed on the allergy risks of genetically modified food crops. Already, ingredients of genetically modified soy and maize are used in many products. Technically, within a few years in Europe a large number of these crops will be introduced on the market (although it should be noted that mounting opposition against these foods may introduce delays of one or more years).

The genetically modified crops that are currently on the market do not contain novel genes that are likely to have an impact on the food allergy situation (some biotechnology critics will disagree!). However, there is a number of genes of interest from an agronomic point of view that must be studied with extra care, before any of those is transferred to food crops. At the workshop, the following proteins were mentioned as potentially allergenic: albumins, lectins and trypsin inhibitors.

Assessment of the allergenic potential

For the assessment of the allergenic potential of genetically modified crops the International Food Biotechnology Council and the ILSI Allergy and Immunology Institute set up a decision tree (see chapter 4). This decision tree, being the only one of its kind, contains a number of tests, which can be used to gather information about the potential allergenicity of the introduced protein: solid-phase immuno-essays, skin prick tests, oral provocation, stability to digestion or processing, amino acid comparisons with known allergen sequences. The outcome of this decision tree is either market introduction of the product without any specific labelling requirement or introduction with labelling as to source of the transgene. All elements of the decision tree can be criticised, in particular, the tests proposed for assessing proteins from a source with no or unknown allergenicity.

In the workshop several modifications of the decision tree were proposed:

- the use of a serum-based test for testing proteins with unknown allergenicity;

- ❑ the use of a well-validated animal model for testing proteins with unknown allergenicity (at present such a model does not exist, but participants felt there was an urgent need for such a model);
- ❑ the source of the test material is of major importance. If the food is consumed fresh, fresh material should be used, if it is consumed processed, processed material should be the norm. Attention should also be paid to the source of the protein if it is used in a purified form. Theoretically it can be obtained from the donor organism, the acceptor (or food) organism as well as a microbial production strain.

Risk assessment and risk management

For assessing the risks of proteins that are mildly allergenic or proteins that are derived from sources with unknown allergenicity, additional aspects must be considered. In these cases, for a proper characterisation of the risk, the following aspects should be considered: allergenic potency (in the sensitisation phase and the elicitation phase), exposure and exposure conditions, prevalence and degree of sensitisation and incidence, nature and severity of effects.

Allergenic proteins will only result in sensitisation or elicitation if consumed above the threshold level of that protein. Therefore, the assessment of threshold values for novel proteins (introduced via genetic modification) and for known allergens is urgently needed. The lowest level of peanut (the most potent allergen) resulting in clinical reactions, could be used as the highest exposure level of a protein of unknown allergenic potential.

For a general market introduction of a transgenic crop of which the transferred protein has limited allergic potential, several risk management options are feasible. It should be preceded by a limited market introduction (place and time). In addition, the products should be labelled indicating the source of the transgene; possible effects should be monitored. In case the allergic potential of the transferred protein is unknown, the worst case scenario should be considered: the transferred protein should then be treated as peanut allergen. If risk assessment shows that the crop is safe, the product could be introduced without any limitations.

Risk communication

Risk communication is seen as a two-way process: not only educating the public about risks, and the risk assessment procedure, but also integration of public participation in hazard and risk assessment and the decision-making process. For proper communication a common language is needed that can be understood by all actors and stakeholders.

Information needs for allergy patients are very different compared to the needs of the public in general (e.g. information about specific allergens).

Labelling is seen as the prime source for informing consumers and patients about new allergenic properties in food. All other information sources (databases, brochures, articles, internet) should be used as well, in particular databases. Information should be unbiased and understandable to the general public.

Many participants, in particular representatives from patients and consumers organisations, stressed that products provided with known allergens should not be allowed on the market. Others disagreed on this point.

1 Introduction

The recent introduction of ingredients from genetically modified crops has brought forward many questions on food safety, environmental safety and labelling. One of the main arguments used by critics is that genetic modification may increase the risk of food allergy. Others point at the possibility of using genetic modification to remove or weaken existing allergens in food organisms

There has been no well established evidence yet of the occurrence of new or more allergies after the market-introduction of genetically modified crops, mostly soybean, maize and rapeseed with novel agronomic traits. However, as the number of genetically modified crops on the market increases, as well as the number of introduced traits, the assessment of the allergenic potential of these foods needs serious attention. A recent example of a soybean variety modified with a gene coding for a Brazil nut allergen, shows that the risk of allergy is not strictly hypothetical.

Until recently the question whether GMO-foods may have additional allergy, has almost exclusively been debated in the scientific domain. In 1993-1995, US representatives of the International Food Biotechnology Council (IFBC) and the ILSI Allergy and Immunology Institute discussed the issue thoroughly. In 1996 their efforts resulted in a decision tree for the assessment of allergenic potential of food derived from genetically modified crops. Since then, no systematic efforts have been made to improve the decision tree.

In the European public domain consumer and allergy patients organisations play a dominant role in discussions concerning biotechnology resp. allergy. However, the issue of biotechnology in relation to food allergy had never been subject of more detailed study. Moreover, these organisations have not been involved in any scientific debate or decision-making process. Therefore, the Consumer and Biotechnology Foundation in co-operation with BEUC, the European Association of Consumer Organisations and EFA, the European Federation of Asthma and Allergy Associations experienced a need to study the issue of biotechnology and food allergy, and organised a workshop. This report is the result of the workshop: Genetically modified foods and allergenicity: safety aspects and consumer information, 28-29 May 1999, Breukelen, The Netherlands.

The over-all aim of the workshop was to give a state of the art of the field and discuss a number of issues, such as the values and flaws of the various testing methods, decision-making procedures, the perception of these risks by the public and the need for information for consumers and patients.

In chapter 2, Harry Kuiper gives an overview of the present and coming generations of genetically modified food crops. In addition, he describes the various safety aspects and the testing systems required. In chapter 3, Carsten Bindslev-Jensen describes the clinical aspects of food allergy. He focuses on the symptoms and diagnosis of food allergy. In chapter 4, Steve Taylor explains the backgrounds of the IFBC/ILSI decision tree, which he co-authored.

Chapter 5 summarises the results of the working session on hazard assessment. In this working session the IFBC/ILSI decision tree was studied and commented. Chapter 6 introduces the issue of how to deal with a given hazard: if the allergenic potential of a new protein is assessed, how do we assess the risk? If

there is a substantial risk, how do we manage it? Chapter 7 deals with public perception and the communication of risks: which forms of communication are needed? What role could product labelling play?

2 What genetically modified foods can we expect?

How do we assess their safety?

Dr. Harry Kuiper, State Institute for Quality Control of Agricultural Products (RIKILT), NL

Allergenicity is one of the items which always comes up when debating the safety of genetically modified foods (GMO-foods). Nevertheless, Mr. Kuiper expressed his doubts whether this issue is specifically related to these foods. He continued by presenting a general outline for the toxicological testing of GMO-foods.

Especially in the US, the area of GMO crops planted is already huge, comparable with the size of the UK. Biotechnology will have a big impact on agriculture world-wide. In addition to food safety, aspects such as usefulness, environmental safety, labelling and traceability are relevant issues.

The key question is if the existing tests and protocols suffice for assessing the safety of GMO-foods. Or do we need new or improved methods? Is there a need for a general framework of testing? Especially given the transatlantic differences in dealing with safety, there may be a need for harmonisation of testing strategies.

First generation of GMO plants

The first generation of food plants is focussed on the improvement of agronomic traits, such as:

- improved disease resistance (viruses, fungi);
- improved pest resistance (lepidoptera, beetles);
- tolerance for herbicides (glyphosate, glufosinate);
- delayed ripening.

These products are already cultivated in large amounts, especially in the US. Almost every crop has already been genetically modified or will be in the near future. Especially crystal proteins from *Bacillus thuringiensis* (Bt), for insect resistance make up a very important class of proteins used for genetic modification. For herbicide tolerance a set of genes is used, of which the genetic construct and the properties are well characterised. In addition a number of antibiotic resistance marker genes is used. Their safety should be assessed on a case-by-case basis. Also antisense constructs should be assessed. Although these do not express new proteins, possible modifications in the plant due to the process of genetic modification as such should be identified.

Mr. Kuiper stressed the relevance of assessing the environmental impact of GMO-crops, aspects such as the build-up of resistance in crops, the spreading of resistance to weedy relatives, the effect on non-target organisms, the possible increase in the use of herbicides, and altered metabolism of pesticides in the GMO plant.

In summary, the first generation of GMO-crops is well characterised. An example is the use of the Bt-proteins, that have been in use as a pesticidal spray. These proteins have sufficiently been tested. However, the long term environmental impact of Bt crops has not been adequately dealt with.

Insecticidal proteins

There are a number of new (insecticidal) proteins which need to be considered carefully, such as: new Bt-proteins (that have not been used as spray);

- proteinase inhibitors;
- amylase inhibitors
- lectins;
- chitinases.

Each category of proteins or individual protein has its own properties in terms of insecticidal efficacy and toxicity for humans and animals. We cannot deal with these proteins in a general way. They should be looked at on a case-by-case basis. Furthermore, work is in progress on other insecticidal compounds. There is a bacterium, *Photobacterium luminescens* with a very ferocious pathogen for nematodes, containing a number of toxins, antibiotics and antifungal compounds, proteases and luciferases, a mixture of potentially toxic products. The toxicity of these genes apparently depends on the expression system used. Deletion mutants of these proteins may be toxic when expressed in plants. In insects, very little is known about the toxicity mechanisms. For these types of proteins, if inserted in plants, extensive safety testing is required.

A new development is that a set of different Bt-proteins, each with a specific insecticidal spectrum, is expressed in one plant. These expression cassettes should also be considered based on our knowledge of the single Bt-proteins. A second trend is the increase of insect resistance by increasing the expression of Bt-proteins in chloroplasts. Obviously this affects exposure to humans and animals.

Safety assessment should be thoroughly and rigid and be focussed at the new proteins. The toxicological consequences of over-expression on chloroplasts should be looked at. Also, the combined expression of proteins should be considered: is potentiation of proteins an issue? Recent literature suggests that the combined expression of Bt-proteins and proteins from other bacteria may potentiate (enforce) insecticidal activity.

The strategy for toxicity testing should be tailor-made, i.e. the protein's biological activity should be taken into account, i.e. activities such as receptor binding or receptor mediated effects and immuno-toxicological and hormonal effects.

Quality traits

The situation will be more complicated in plants with novel quality traits. Examples are:

- improved quality of seed storage protein (e.g. by increasing the methionine content);
- higher starch content, 'waxy' starch and novel carbohydrates;
- better oil quality;
- fortification with micro-nutrients (e.g. iron) and antioxidants;
- reduction/elimination of allergens and natural toxicants;
- increase absorption of critical nutrients;
- carrier for edible vaccines.

As an example iron-rich rice has been developed. This may be interesting for those that do not consume sufficient iron. However, would it be helpful for healthy people?

Another example are plants enriched with beta-carotene's for their antioxidant activity. However, longterm intervention studies with β -carotene were discontinued at an early stage, as negative effects were measured as well.

Thus, more research is needed regarding the presumed beneficial effects of certain compounds, preceding the development of plants with specific health claims.

We must make sure that in testing these products, we should tailor our testing to the modification made.

Another area is the production of antigens in plants, e.g. potato and tobacco, that can provoke antibodies against diarrhoea and diabetes.

Testing systems

The main question, do we have the appropriate system, to test the safety of these products, can only be answered on a case-by-case basis. The safety evaluation of these types of product cannot be carried out based on the concept of substantial equivalence. It can be that we do not have the parent lines of the plants available. The composition of the plants may have changed dramatically. So, we need much more rigid testing than we need for GMO-plants from the first generation. We need to design protocols consisting of compositional analysis of these new plants, and a combination of animal studies, in vitro-studies and possibly human volunteer studies. The kind of testing of whole, complex foods cannot be compared with testing a single chemical compound, where existing protocols have been standardised. In testing whole foods, there are many confounding factors, e.g. in the composition of the diet. The experiment may not be sensitive enough. If we can avoid feeding studies with foods, we should do so. Testing the isolated expressed proteins of the transferred genes as well as the use of in vitro-test systems has to be favoured. In addition to the testing systems mentioned, there are other ways of studying unintended effects. These can be looked upon on various levels. One can e.g. study:

phenotypic characteristics,

- DNA patterns,
- messenger-RNA profiles using the micro array hybridisation technique
- protein profiles, using the proteomics technique
- metabolite profiles.
- There is a number of quite sensitive techniques available. You can e.g. compare metabolite profiles of the genetically modified plant with the one of the non-modified plant. If differences are found, this may indicate that further toxicity is required.

Conclusions

For the testing of the new generations of GMO-plants, a combination of various techniques and methods is needed. These methods must be selected based on the type of genetic modification. Especially animal testing should be further developed and refined. Mr. Kuiper expressed his doubts about the development of plants expressing compounds that have so-called beneficial health effects, as too little is known about their mechanisms of action, the long-term effects and safety margins of these components. Substantiation of these health effects claims needs a lot more work. The problem is that the biotechnologists proceed very quickly, while the toxicologists lag behind. And last but not least, new techniques to trace unintended

effects should be further developed. This will hopefully strengthen public confidence in the safety and wholesomeness of genetically modified foods.

3 Clinical aspects of food allergy

Dr. Carsten Bindslev-Jensen, , Odense University Hospital, DK

Mr. Bindslev-Jensen stressed the importance of double blind placebo controlled food challenges (DBPCFC). If one is not prepared to carry out DBPCFC in patients with suspected food allergy, or does not have access to a centre where these tests are carried out, one should not deal with these kind of questions.

In children below the age of 7 the over-all incidence is about 7-8%, both in Europe and the US. There are firm data on cow's milk allergy but for the rest the data are very scarce. In addition, 1-2% of the children has non IgE-mediated response to additives. The prevalence in adults, confirmed by double-blind placebo-controlled food challenge (DBPCFC) is lower, at the most 1%.

It is IgE-mediated reactions that may kill people. Many fatal cases concern peanut allergy. As in Europe, more and more peanut ingredients are consumed, the more peanut allergy we found.

Anamnesis and symptoms

We do not know anything about how people get sensitised. It is e.g. not known whether sensitisation takes place through the intestinal tract or via inhalation. What we do know is what to do when we have the case history of a patient or in case he claims he has an allergy. There is some difference between assessing allergy in adults and small children, but in the end it comes down to the same system: take a thorough case history, do the appropriate diagnostic tests, put the patient on a diet (sometimes an elimination diet) and end with DBPCFC. The only difference is that in small children a food challenge is not always possible, while in adults, one has to carry out a DBPCFC.

The first symptom is often (in 90% of the patients) Oral Allergy Syndrome (OAS). Immediate contact of the food with the mouth's mucosa causes itching, which can be very intense. In fact, this is a natural warning system for the allergic patient. Then it is followed by a second reaction, the systemic reaction. Normally, patients now have concomitant symptoms in more than one organ system. That means that very few patients will respond with only atopic dermatitis. Very few patients that only have asthma, are food allergic. So, we should look for patients that develop acute systems in the gastro-intestinal tract and/or the respiratory tract and/or the skin. These patients have a high probability of giving positive results in the food challenge.

The time course of food allergy is very typical. Normally there is an immediate reaction, ranging from itching in the mouth, gastro-intestinal disturbances to asthma. There may be late-phase reactions 24 to 48 hours after ingestion of the food. Patients having atopic dermatitis deteriorate 24 hours after the challenge. But these patients - as far as I have seen them - also have an acute phase with e.g. gastro-intestinal symptoms. Patients, only having a response after 24 hours or more, are very rare.

Diagnosis

How do we select the food that causes the allergy and which should be avoided by the patient? The ideal diagnostic test should have 100% sensitivity (the ability to detect all the diseased patients) as well as 100% selectivity (the ability to exclude all the non-sensitive patients) does not exist. Another issue is the amount of specific IgE that should be present. There should also be data on the range of specific IgE which is normally present in healthy individuals. In addition we should have testing systems without cross-reactions. A test for e.g. grass pollen should not pick up IgE for wheat. However, at present, a test meeting all these requirements is not available.

One test comes very close to the ideal test, the test for the major codfish allergen. The allergen is very stable for digestion. The test has very high sensitivity and specificity. In the case of hazelnut: 70% of patients that respond positively after challenge would be negative in the *in vitro* assay. This would mean that these patients should probably be classified as hazelnut intolerant instead of allergic. Hazelnut allergic means that a patient has a positive challenge and specific IgE. However, if one takes fresh hazelnut, many patients that previously tested negative will now test positive. Apparently, the difference between classifying a patient as either hazelnut allergic or hazelnut intolerant depends on inappropriate (i.e.: not fresh) abstracts.

The test systems for wheat are very sensitive but not very specific. At present we cannot discriminate between grass pollen and wheat. An assay for apple appears to be 100% specific but the sensitivity is zero. This is because there is too little allergenic protein present in the assay. This means that all the patients that have OAS after eating an apple are intolerant according to the assay. However, in the skin prick test these patients are apple allergic. So, the usefulness of a testing system depends on the patients, the time available for testing the patient, the quality of the test and your interpretation of a positive or negative test result. A testing system which is good for codfish is not necessarily good for egg or apple. In case of a positive skin test or RAST assay, a food challenge may still be negative. This may be due to the many clinically insignificant serological cross-reactions, where IgE raised against and directed towards epitopes on, for example, grass pollen, also bind to wheat proteins, but without any clinical significance of the finding

The types of *in vitro* testing systems in use can be divided into three categories:

1. Thoroughly validated tests (DBPCFC and specific IgE): includes assays for codfish, cow's milk, hens egg, shrimp, soybean and, recently, wheat and hazelnut;
2. Well validated tests (specific IgE)
3. Tests that have the benefit of the doubt, such as measuring specific IgG.

At the moment we have ongoing protocols of validation of tests against DBPCFC. Both, the sensitivity and specificity of these tests are improving. We have not solved the problems with cross-reactivity so far.

Patients that are going to be tested in a food challenge have to be put on a diet for some time, in order to reduce any symptoms before the start of the challenge. The DBPCFC is the only way to confirm the diagnosis. However it is by no way perfect and there are many unsolved questions surrounding this test. It is also very relevant to what amount of allergen a patient responds. It is very relevant if a patient responds to 50 grams of the food or just 50 milligrams.

Also, there have been DBPCFC studies in which many patients reacted to the placebo. This shows that if one has dubious results such as these, the challenges should be repeated. Another issue is what to do with these placebo reactions. In many papers, the results of patients reacting to placebo's are left out. This is a mistake: if the responses to the test substance and the placebo are similar, there is no connection between the challenge with the foodstuff and the symptom. In summary: here is a need to standardise the whole procedure for DBPCFC, including the use of placebo's.

In conclusion, if someone is allergic for a foodstuff, it is very relevant to find out how they established the diagnosis. Was DBPCFC used? How sensitive and specific were the assays? What was the precision?

Genetically modified foods

Concerning GMO-foods, establishing allergy here it is just as difficult as in conventional foods. Firm, solid data from patients should be looked at in order to be able to help them. These same data are needed in order to help manufacturers, governments and the public to find out whether these GMO-foods cause any additional problems.

4 Allergenicity of foods produced by genetic modification

Prof. dr. Steve Taylor, University of Nebraska, US

Mr. Taylor has been on the IFBC/ILSI panel that made the decision tree for the assessment of the allergenic potential of genetically modified food crops¹. In addition he has chaired the FAO/WHO Expert Consultation on Biotechnology and Food Safety, Rome, 1996². Food allergy was one of the major issues at this Consultation.

Food allergy and allergens

The prevalence of food allergy: 1-2% of adults and 4-6 (or 8) % of children suffer from food allergy (i.e. IgE-mediated immunologic reactions). Eight groups of foods, the so-called 'big eight' account for over 90% of allergic reactions and adults and children. This includes crustacea, egg, fish, milk, peanuts, soybeans, tree nuts and wheat. The symptoms are highly variable, ranging from mild discomfort to systemic anaphylactic shock. In addition to food allergic patients there are individuals that suffer from gluten sensitive enteropathy (celiac disease). These other reactions, cell-mediated reactions are not covered by the decision tree. However, these diseases are manageable and should not be ignored.

Virtually all food allergens are proteins but not all proteins are food allergens. Foods in general contain hundreds of thousands of proteins, but few of them are allergens. Many allergens are stable to digestion and processing. Especially major allergens (allergens that account for over 50% of the allergic responses in sensitive humans) tend to be abundant proteins.

Proteins and biotechnology

In comparison with traditional plant breeding, genetic engineering precisely controls which proteins are introduced into organisms. Most proteins introduced into crops are enzymes that are not stable to digestion or processing. Most applications require only the expression in plants of minute amounts of new protein (e.g. insect resistance) or no protein at all (e.g. antisense technology for delayed ripening). Biotechnology does hold the promise to reduce the allergenicity of foods. If e.g. the allergenic potential of peanut allergen could be reduced through genetic modification, this would be a tremendous advance. If one could reduce the allergenicity by 50%, the number of people that become sensitised would be reduced considerably.

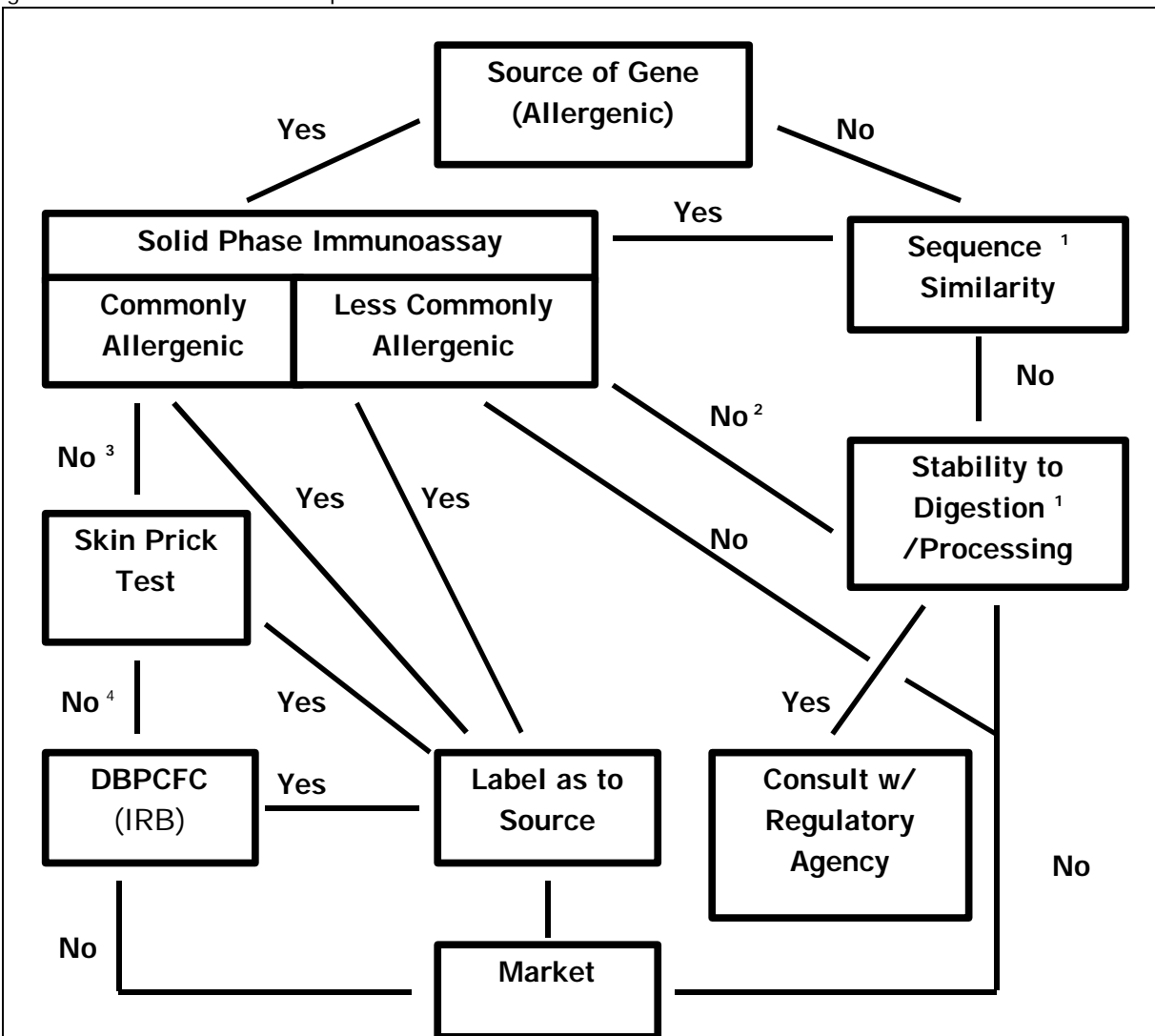
Scope of the IFBC/ILSI-report

Already in late 1993 IFBC and Allergy and Immunology Institute (AII) of ILSI started having meetings on the issues of biotechnology and food allergy. At that time there was no framework whatsoever. The job was

¹ Metcalfe, D. *et al.* (1996) Assessment of the Allergenic potential of foods derived from genetically engineered crop plants. In: *Critical Reviews in Food Science and Nutrition* **36**, S165 - S186.

² FAO/WHO (1996) Biotechnology and food safety - report of a Joint FAO/WHO Consultation, Rome, Italy, 30 September - 4 October 1996. FAO Food and Nutrition Paper 61.

to develop the best framework possible. IFBC is a group of individuals from various genetic engineering companies who have an interest in this area. The All of ILSI is composed primarily of academic and governmental scientists and specialists



¹ It is recommended that an assessment for amino acid sequence similarity to all known allergens and an assessment of stability to digestion be performed for all gene products.

² Solid phase immunoassay tests depend upon availability of sera. Ideally, 14 sera should be used. However, if less than 5 sera are used, then proceed to stability box if results are negative and consult with the appropriate regulatory agency.

³ In the case of equivocal results or suspected false positives, proceed to skin prick tests.

⁴ DBPCFC's are performed on food products in which there is no evidence of allergenicity based upon solid phase immunoassays and skin prick tests. To assure lack of allergenicity, DBPCFC's should be performed following IRB approval.

Figure 1: The IFBC/ILSI decision tree for the assessment of the allergenic potential of foods derived from genetically engineered crops (1996)

in allergy. The group consisted of US-based allergologists, food scientists and biotechnologists. The report¹ covers the following items:

- cellular biology, symptomatology and treatment of food allergy;
- catalogue of allergenic foods;
- characterisation of the major food allergens;
- tests for food allergens;
- assessment of the allergenic potential of foods derived from genetically engineered crops.

The group focussed on plants and methods of genetic modification for food crops.

As part of the process the IFBC/ILSI group performed an extensive search in the medical literature for reported allergenic foods. The University of Nebraska maintains a database for most of the published literature on adverse reactions to foods. Using this database, a table of allergies to over 160 different foods was composed.

The decision tree for the assessment of the allergenic potential of foods derived from genetically engineered crops was jointly developed by IFBC and ILSI experts (figure 1). Its strategy is based on the gene source, sequence homology with known allergens, immuno-chemical analyses and physicochemical properties of the expressed protein. It should be stressed that not one of these methods by itself would be very useful or fully predictive: it is the totality of assessments which provides reasonable assurance that foods will not be rendered newly allergenic.

Safety evaluation

According to the decision tree, as a first step one has to determine if the gene is isolated from:

- a commonly allergenic food;
- a less commonly allergenic food;
- a source with no allergenic history.

If the source of the gene is from an allergenic source, the left-hand side of the decision tree applies, if it is from a non-allergenic source the right-hand side applies. Mr. Taylor explained that he is very comfortable with the left-hand side of the decision tree. The order of test is such that the most invasive test (food challenge) is only required if other tests are negative. It is a prerequisite that the patients used are very well characterised.

The right-hand side of the decision tree contains tests such as amino acid sequence homology and digestion testing. Those tests can be criticised. However, in 1993-1995 no better tests were available, and, this is still the case.

All of the genes that have been transferred to plants which are currently commercialised fall into the category of 'derived from sources without allergenic history'. It is recommended to compare the amino acid sequence of the proteins of all transferred genes with sequences of known allergens. If there is

homology of at least 8 contiguous amino acids (the smallest length of a continuous T-cell binding epitope), the protein could be an allergen. From databases such as GenBank, PIR, EMBL and SwissProt, the amino acid sequences of over 198 known food and other allergens can be retrieved, using FASTA or similar computer programmes. This work can be done very quickly, in less than an afternoon.

The left-hand side of the decision tree is the way to go, anything short of this would put allergy patients at risk. Many of the large biotechnology companies, at least in the US uses these approaches. However, in the literature cases can be found in which this approach has not been considered. An example is an article in Nature in which a Japanese group increased the iron content in rice by the introduction of a soybean protein. However, there was no mention of soybean allergy in this paper, while soybean allergy is the most common allergy in Japan. The Japanese group should be aware of this and carry out appropriate evaluation studies.

Commonly allergenic donor

According to the left side of the decision tree (known allergens) a minimum of 14 patients sera has to be used. This number gives over 99,9% probability of detecting a major allergen and over 95% probability of detecting a minor allergens to which over 20% of the sensitive population reacts. In case the number of 14 patients cannot be found, at least 5 test sera result in over 95% probability of detecting a major allergen. Mr. Taylor indicated he did not feel comfortable with this small number of 5 sera. As an example, finding people with sunflower seed allergy is very difficult, even finding 5 is difficult.

The left hand side of the decision tree was used for assessing the allergenicity of the 2S Albumin storage protein from Brazil nut, which was expressed in soybean. This work was done by Mr. Taylor's laboratory. Soybeans are deficient in methionine, while Brazil nut is very rich. The Brazil nut storage protein contains 18% methinone, the highest methionine content known. Therefore, expression of this protein in soybean increases the methionine content, which is especially useful for the chicken feed industry.

At forehand, the various allergens in Brazil nut had never been studied, so the Brazil nut allergen was unknown. As co-mingling with soybeans for human nutrition could not be excluded, the question arose whether the Brazil nut 2S albumin was an allergen. This was tested by testing 9 sera from Brazil nut sensitive patients against extracts of the transgenic high methionine soybean. The pooled sera gave a positive RAST (RadioAllergo Sorbent Test) and 8 out of 9 patient sera reacted positive to the introduced protein on immunoblots. Skin prick tests on three Brazil nut sensitive patients confirmed the presence of the allergen. A food challenge was not carried out as this would be too dangerous³.

Pioneer Hi-Bred, the company which had developed the product (and paid for the research) decided to discontinue further product development.

Biotechnology can also lead to decreases in allergy content. Matsuda in Japan was able to develop transgenic varieties of rice with less of the allergen. Unfortunately, many of the patients used in the Japanese research were not actually sensitive to ingested rice. This research may be the result of grass-related allergens in rice.

³ Nordlee, J. *et al.* (1996) Identification of a Brazil-nut allergen in transgenic soybeans. *The New England Journal of Medicin* **334** (11), 688-692.

Recently, researchers of the CSIRO-institute, Australia, introduced a gene from sunflower in lupine and alfalfa, with a similar objective as in the Brazil nut gene case. A very limited number of sunflower allergy patients is known, and apparently the characteristics of the expressed sunflower protein are quite similar to the Brazil nut 2S protein. Taylor's group proved that this sunflower protein is an allergen, by showing that three sunflower allergy patients reacted positive to the protein. Mr. Taylor advised CSIRO to stop developing the transgenic product.

Less commonly or unknown allergenic donor

In case of a less commonly or unknown allergenic donor, the decision tree recommends amino acid comparisons with known allergens and the testing of digestion rates. Most allergens or fragments are resistant in simulated digestion models, while most proteins expressed by transferred genes are not. Mr. Taylor underlined this by showing a number of digestion tests done by Monsanto on a variety of plant proteins, allergens and non-allergens. In case the whole protein of an allergen is not very stable, the fragments usually are.

Mr. Taylor expressed his doubts about the right hand side of the decision tree. What do we do e.g. in case of a protein from a non-allergenic source, but stable to proteolysis and digestion? At present this part of the decision tree is all we have and it should be regarded as a work in progress.

In addition to proteins from less commonly allergenic sources, proteins from sources with no history of allergy are already on the market or will soon be marketed. These proteins do not have amino acid sequence homology to known allergens. All proteins transferred hitherto are rapidly digested. Further, introduced proteins are expressed at low levels relative to the major allergens. Mr. Taylor concludes that there is no allergic concern with these products. However, this is not to say that future products are of no concern.

Conclusion: IFBC/ILSI decision tree approach

The *totality* of these assessments provides reasonable assurance that foods derived from bioengineering will not introduced allergenic concerns beyond those that already exist in the food supply.

Mr. Taylor is in favour to add decision points to the decision tree if these are validated. In the future, the integration of animal models should be considered. However, the use of a non-validated animal introduces more problems than it solves.

5 Working session 'Hazard assessment'

Moderator: Prof.dr. Paul Davis, Unilever, UK

Introduction: Dr. Wolf-Meinhard Becker, Borstel Research Centre, BRD

Report: Frans van Dam, Consumer and Biotechnology Foundation, NL

5.1 Introduction:

Hazard assessment of the allergenic potential of genetically modified food

IFBC/ILSI decision tree

The decision tree of IFBC/ILSI is an appropriate starting point to discuss the hazard assessment of the allergenic potential of genetically modified foods (Metcalf *et al.*, 1996; see figure 1). The left-hand side of the decision tree is indisputable in the case of introduction of allergenic components and in the case of the 'Yes-arrows'. Here, an allergenic hazard is clearly given.

The key question is: If all results of testing methods are negative, can the absence of allergenicity then be established? At present this question can not be answered, because there are too many gaps in our knowledge about the patho-mechanism of type-I-allergy. It is e.g. hypothesised by some that the protease activity of some allergens may interfere with receptors for IgE, thus inhibiting the suppression of IgE synthesis.

Negative test results are necessary but not sufficient to prove the absence of allergenicity of the introduced protein. But if a 'reasonable amount of certainty' rather than a 'sufficient condition' is the standard, how many negative test results according to allergenicity do we have to collect to meet it?

Testing methods/ diagnostic tools

The order of the tests proposed by the IFBC/ILSI is correct; because of ethical reasons, *in vivo* tests should be used in the proposed order if these cannot be avoided. The diagnostic sensitivity of solid face immunoassays is on an average 70%. Thus, 30% of the patients give false negative results, which may be caused by the quality of the extract or denaturation of the allergens in question by the immobilisation procedure etc. This clearly demonstrates, that the test systems for the introduced protein must be evaluated. This implies at least positive control sera - raised in animals - to the introduced protein. The aim of identifying the introduced protein as minor allergen (20% prevalence) by 14 down to 5 sera is rather unsatisfactory since 20% prevalence is too high as minor level.

Why is the pure protein not used to screen its IgE-reactivity by patients' sera? This can and must be done world-wide, when global marketing of the transgenic food is desired. Moreover, this would have the advantage, that more test sera are available. In addition, sera of patients with a different sensitisation pattern or sensitisation background are obtainable.

IgE reactivities of two groups of allergic patients should be tested:

1. Sera from patients who are allergic to the allergenic source material from which the introduced protein is derived (also sera from patients that only respond to minor allergens of a given food, should be used in assessing the allergenicity of a protein derived from that food);
2. Sera from randomly selected allergic patients in order to detect cross reactivities.

In Mr. Becker's opinion, the food industry should refrain from transferring components of allergenic source material to the edible parts of food plants. This might be supported by the fact that allergic diseases and especially food allergies increase in the population, which may be accompanied by a parallel increase of IgE specificity's to known allergenic source material.

Physicochemical properties

Many allergens of fruits and vegetables causing oral allergy syndrome are labile during processing, whereas 'classical' food allergens from egg, cows' milk, soybean and peanut are at least partly stable. The stability of IgE reactivity during heating and processing can therefore indicate potential allergenicity, but absence of stability does not exclude allergenicity of the protein.

The location of sensitisation to food allergens is unclear at present. Apart from the lower gastrointestinal tract, the respiratory tract and the tonsils as first lymphoid organ of the gastrointestinal tract are discussed as the place of sensitisation. Oral contact with peanut in allergic patients, without swallowing, resulted in immediate symptoms.

The assessment of stability of a protein to digestion in simulated gastric fluid in the described experimental design has no sufficient predictive value. In these studies matrix effects and the generation of immunogenic fragments are not taken into account. Here, the TNO gastro-Intestinal Model (TNO-Nutrition, Zeist, The Netherlands) may be a suitable, experimental basis to investigate these aspects in vitro.

On the one hand it is evident from hypo-allergenic infant formulae that allergenicity is reduced by partial and extensive hydrolysis, on the other hand formulas can cause severe type-I-reactions in nearly 50% of sensitised infants, and even elicit sensitisation. Moreover, food processing can also result in the formation of new allergenic determinants. Increased allergenic reactivity after heating has been determined e.g. in molluscs, and in pecan nuts. Such new determinants have also been formed by chemical reactions between soybean oil and proteins or via Maillard reactions between lactose and milk proteins.

In summary, stability testing during processing and digestion can supply useful information, but extended experiments are required taking into account the complexity of sensitisation and triggering of symptoms in food allergy.

Amino acid comparisons

Similarity searches, comparing novel food proteins with sequences of known allergens, can be indicative of an allergenic potential. However, the major criterion for excluding an allergenic material is the assumption that 'immunologically significant sequence identity requires a match of at least eight contiguous identical

amino acids'. Although this corresponds to the minimal size of T-cell epitopes, it is not sufficient to define immunological significance. The following examples may disprove the above statement on B-cell level.

It appears that the major codfish allergen contains 2 tetra-peptides which are essential for IgE binding, separated by a 6-residues spacer, which is irrelevant for IgE-binding. A second example is the major peanut allergen, with a minimal epitope size of 6 consecutive residues.

The authors of the IFBC/ILSI decision tree also state that *'this approach is limited in that it cannot identify discontinuous conformational epitopes'*. This is true but it represents an important pitfall since protein structures and not sequences are being conserved due to a common structure-based function.

IgE cross-reactivities could be shown between birch pollen profilin and human profilin. In this case, the longest stretch of matching amino acids contains 4 residues. Despite an overall sequence identity below 30%, the tertiary structures and functions of both proteins are very similar, showing that structure-based cross-reactivity can easily be overlooked.

Finally, it has to be noticed that small peptides of 3-4 residues can resemble discontinuous epitopes. These so called 'mimotopes' are again not covered by the approach of the decision tree.

Thus, for a more reliable safety assessment it is suggested that the level of matches be reduced to four identical consecutive residues. In addition, overall similarities should be calculated from end-to-end alignments to identify proteins with homologous structures.

Animal models

Most animal models in type-1-allergy research have neither been developed nor validated for assessing the allergenic potential of specific proteins in humans. One of the major problems in using animal tests is the variability in responses from allergen to allergen, animal to animal and species to species. However, it should be noted that the variability of responses of different individuals to identical stimuli is a characteristic feature of allergy as well as general human toxicology. This inherent complexity should hence not inhibit the development of models for type-1-allergy with particular consideration for allergy to food. There is evidence that some animal models might be useful to predict allergenic potential. These models should be further developed and tested with known allergenic as well as non allergenic proteins as quickly as possible.

Conclusions

1. Similarity searches with known allergen sequences have to be based on a minimal match of 4 identical consecutive amino acid residues when considering cross-reactivity and B-cell level. These should be supplemented by end-to-end alignments. In the near future structure prediction models could be included in an extended procedure.
2. Immunogenicity of new food proteins and their digests should be tested by immunising animals. The resulting sera could be used to monitor the digestion process.
3. Monitoring of the IgG response to novel proteins in man after market introduction of genetically modified foods is recommended.

4. Parallel to market introduction, field studies of food allergies should be undertaken in selected matched patient groups consuming and avoiding genetically modified plant foods.
5. Similar to sequence identity, IgE-inducing potential in specific animals could be used as an indicator but not as a determiner of allergenic potency.
6. The potential of newly introduced food proteins to cause respiratory sensitisation should be studied in occupationally exposed persons.

5.2 Outcome of discussions

The hazard assessment session was concerned with identifying and characterising all those factors that could possibly contribute to an allergenic hazard of a transgenic protein expressed in a GMO food plant, as well as testing methods. The session was not concerned with defining the risk aspects (i.e. the chance, great or small, that someone will be harmed by that hazard).

To ensure that no factor was missed, the participants conducted a very thorough review of all those factors that could make a transgenic protein behave as an allergen. Initially this was done independently of the IFBC/ILSI decision tree (Metcalf et al., 1996), to ensure that the group's thinking was not too constrained. The IFBC/ILSI document was then analysed and challenged in the light of the group's findings, to arrive at an independent and thoroughly credible view.

Factors relevant to the assessment of the allergenic potential of GMO food crops

The views of the group are as follows:

1. *The properties of the expressed protein*

- ❑ known allergenic characteristics of the protein : Obviously, if a protein is known to have allergenic properties, this is the starting point for further studies and / or decision-making;
- ❑ antigenicity of the protein: Proteins that are highly antigenic / immunogenic (potential to evoke specific IgG and/or IgE) are more likely to cause allergies than proteins with low antigenicity. Although the presence of specific IgE in the serum is a prerequisite for IgE-mediated allergy, not all individuals having specific IgE, exhibit symptoms after consumption of the product. Every non-human protein has at least some antigenicity in humans. Proteins expressed by transferred genes will probably always have some level of antigenicity. The participants felt that we should not demand that a protein be less antigenic than general food proteins;
- ❑ stability under processing and gastro-intestinal conditions: The best known allergens, such as peanut allergens are relatively stable to processing and gastro-intestinal conditions (e.g. proteolytic enzymes and low pH). However, some apple allergens are known to be very unstable in these conditions, but still cause oral allergy syndrome. It was also noted that the texture of the foodstuff may protect the protein from degradation. The participants felt that the lack of stability of the expressed protein does not guarantee that the expressed protein is not an allergen. Nevertheless, it is an important factor to consider, especially in relation to the food matrix or texture. In addition, thermal processing can introduce new allergenic epitopes and there are cases (though rare) of individuals being allergic only to cooked or stored food, even though they are completely tolerant of the raw form;

- ❑ cross-reaction with allergenic proteins / amino acid homology with other proteins: If the expressed protein cross-reacts with antibodies in sera from patients that have allergies to other proteins or foodstuffs, there may also be cross-allergy. Similarly, amino acid homology with known allergenic proteins may indicate that the protein has an allergenic epitope(s). However, the minimum number of amino acids to be compared is subject to debate, as was shown in Dr. Becker's contribution. The participants felt that, in addition to cross allergy or amino acid homology with food allergens, non-food allergens, such as dust mite should also be taken into consideration;
- ❑ carbohydrate residues / posttranslational modifications: Glycosylation patterns of proteins may influence antigenicity and possibly allergenicity. As an example, the enzyme amylase expressed in tobacco reacted with specific antiserum, while the same amylase expressed in a bacterial system did not. Epitope recognition can depend of the glycosylation pattern of the epitope(s). The participants felt that the choice of the source of the expressed protein to be tested against reference sera is a crucial factor.

2. *Factors affecting the expressed protein*

- ❑ location of expression : Exposure through the gut requires expression in the edible parts of the plant. If the protein is expressed in pollen, exposure (and thus sensitisation and/or provocation) through inhalation is also possible;
- ❑ interaction with other components: There is a possibility that the interaction of the expressed protein with other agents leads to increased allergenicity. E.g., the introduction of genes for trypsin inhibitors could result in inhibition of proteolysis of allergenic proteins present in the foodstuff during processing or storage; Maillard reactions between milk proteins and lactose can result in new allergenic determinants;
- ❑ absorption across the mucosa: An ingested protein must be absorbed across the mucosa before it can evoke an allergic reaction. It need not be absorbed as a completely intact molecule, but it must be in fragments large enough to display at least two epitopes in a such a way as to cross-link IgE antibodies on mast cells (in the sub-mucosa etc.) or on basophils in the circulation. There are many host factors that influence absorption of proteins and large fragments of proteins, especially the rate of digestion, and the state of gut inflammation (which is more to do with risk assessment);
- ❑ expression levels of the protein and exposure levels: The expression levels of the protein and the exposure of consumers to the protein do not determine the allergenicity of the protein. However, these factors are relevant in the assessment of the risk that a given protein may lead to sensitisation or provocation in patients. The participants assumed that in the working session on risk assessment these factors would be considered in further detail.

3. *Possible influence of the insertion of the transgene on other, endogenous genes*

- ❑ pleiotropic-like effects and other gene interaction effects: After insertion of the transferred gene in the pant genome, its promoter / enhancer sequence could influence the expression of endogenous genes, or genes could be interfered with in other ways (eg to disrupt a metabolic pathway by damaging a gene encoding a vital enzyme, allowing intermediates to build up). These mechanisms might cause proteins that are normally absent or present in trace quantities, to accumulate and thereby contribute to the allergenic potential of the food organism.

Conclusions

1. Most participants agreed that the hazard associated with the genetically modified crops that are currently on the market is acceptably low. Most of the transferred genes expressed in these crops are derived from non-allergenic, non-food, bacterial sources. Moreover, to date, there have been no reports of allergies related to ingestion of expressed proteins in these genetically modified foods. However, in the future, new genetically modified crops may be provided with genes that need special attention from an allergenicity point of view.
2. Examples of potentially allergenic proteins are:
 - ✓ 2S albumin type of storage proteins from plants;
 - ✓ lectins;
 - ✓ domains from known allergens;
 - ✓ (plant) defence proteins, such as enzyme inhibitors.
3. There was agreement that the use of a decision tree is the appropriate way for deciding which tests should be carried out.
4. There was agreement that no single test in the IFBC/ILSI decision tree is good enough to replace the decision tree as a whole. Every single test has its shortcomings.
5. Most participants were satisfied with the left side of the IFBC/ILSI decision tree (concerning expressed proteins from known allergenic sources), provided that the test sera used are representative of the whole allergenic population, and that the allergies of the patients involved are characterised thoroughly. In solid phase immunoassay, large numbers of false-positives and false-negatives should be taken into account. However, a minority disputed that the left side of the decision tree should exist, stating that proteins derived from an allergenic source should never be expressed in food organisms.
6. Some doubts were expressed concerning the right side of the IFBC-ILSI decision tree (expressed proteins from non-allergenic sources). In particular, the rejection criterion of stability to processing and digestion does not exclude all allergens, as some, such as apple allergens are highly labile. Moreover, a minority of the population has low gastric acid levels. In these individuals low pH proteolytic degradation may be severely weakened. It was also noted that most stability tests are oversimplified. The pure protein is tested in isolation and matrix / texture influences are not taken into account, thus resulting in maximum degradation of the protein.
7. At present, no suitable or well-validated animal model is available. The participants agreed that the right-hand side of the decision tree would benefit from such a model. Such models should be developed as soon as possible. A minority had the opinion that no genetically modified food crops should be approved before a well-validated animal model is available.
8. The participants suggested consideration of a serum-based test integrated in the right-hand side of the IFBC/ILSI decision tree. Such a test could consist of testing the protein with a number of sera from different and well-characterised patients, covering a large variety of allergies. Any positive reaction would indicate cross-reaction with known, common allergies. However, there was robust debate about the practicalities and relevance of this.

9. The participants also considered post-market monitoring of genetically modified foods. Screening for the presence of specific IgG was proposed as a marker for exposure (not for allergenicity) to the protein after marketing. It should be noted that not all consumers will respond by making IgG responses.
10. A relevant issue that needs further consideration is the source of the test material. Should this material be derived from fresh foods or from processed foods? In some cases, e.g. apple allergens, the use of fresh material is recommended. If foods are consumed only in a processed form, testing of the processed food should be the norm. If the protein is tested in a purified form, should it be a requirement to isolate it from the genetically modified crop? Or can we use samples of the same protein produced by a bacterial strain, for example?

6 Working session 'Risk assessment and risk management'

Moderator: Dr. André Penninks, TNO-Voeding, NL

Introduction: Dr. Geert Houben, TNO-Voeding, NL

Report: Dr. Margreet Bloemers, Consumer and Biotechnology Foundation, NL

At first, the distinction between *hazard assessment* (i.e. characterisation of the allergenic potential of GMO food crops), and *risk assessment* (i.e. assessing the risk that GMO food crops are indeed able to sensitise and elicit an allergic response) was discussed. Having assessed the risk, it is possible to define measures for risk management to control risk. Subsequently, it is up to the regulatory authorities to decide whether the risk and the management measures are acceptable, and whether the GMO food crop can be introduced on the market.

6.1 Introduction: From hazard assessment to risk assessment

Allergenicity

Allergenicity is the potential of a protein to sensitise and/or to elicit allergic effects in sensitised subjects. For the hazard assessment with respect to the allergenic potential of foods derived from genetically engineered food crops, the ILSI/IFBC decision tree is most frequently used. This decision tree was discussed in session 1a. Risk assessment starts with an alert from the IFBC/ILSI decision tree, indicating that the GMO food crop is potentially allergenic. It was noted, however, that the ILSI/IFBC decision tree is not conclusive in all situations, especially in case the gene to be transferred is derived from a source with no allergenic history or no history of food use at all. A negative result based on the listed tests does not guarantee that the genetically modified food crop is not allergenic. It also does not exclude whether the food is a weak or a strong sensitiser or whether it will show severe or less severe reactions in the elicitation phase in sensitised individuals. It merely indicates that the available tests, in case a gene is transferred from a source with no allergenic history or food use at all, are not able to show potential allergenicity. In fact, Mr. Houben stated, all (glyco)proteins are potentially allergenic. Although the allergenic potential of the vast majority of proteins is very low, it is not possible to totally exclude allergenicity.

Risk assessment

The fundamental question of this working session was: assume that the outcome of the hazard assessment is that the GMO food crop is potentially allergenic, can we imagine that there are conditions under which we can still introduce it on the market?

Allergenic potency

Allergenic potency is a quantitative parameter to rank the potency of an allergen to sensitise or elicit (provoke) allergic effects in sensitised subjects. This ranking can at the moment best be performed relative to the potency of known allergenic food proteins. Future animal models and / or supplementary approaches may provide the necessary tools for the establishment of the relative potency (in sensitisation

and elicitation phase) of food proteins. In general the food has the characteristic to be strongly or weakly allergenic. However, also other factors play a role, such as the immunologic traits of a person and the conditions of exposure to the food. In considering the allergenic potency, one must distinguish the sensitisation phase from the effect elicitation phase.

Sensitisation phase

The prevalence of sensitisation is determined by the sensitising potency of the food, together with various factors determining the exposure to it, like frequency, dose and conditions of exposure.

In case a gene is transferred from an allergenic source, sera of allergic patients to that source are available, and the relative allergenic potency of the transferred protein (coded by the gene) can be determined compared to the other allergenic proteins from that source. Moreover, the allergenic source used for the gene transfer can in addition be ranked relative to the potency of other known food proteins. (e.g. peanut versus potato). However, for newly engineered proteins, or proteins with no history of use in food, antisera are not available. Moreover, at present, there are no validated test systems to assess the sensitising potency of unknown proteins. It is expected that in the future, animal models will prove to be valuable for such tests. At present, only the analysis of physical-chemical properties (e.g., stability of the protein during processing and digestion) gives some clue about allergenicity of unknown food.

Elicitation phase (effector phase)

The incidence, nature and severity of possible adverse events in sensitised individuals upon challenge exposure are determined by the allergenic potency of the food together with the prevalence of sensitisation, and the dose and conditions of exposure. For risk assessment, also the determination of the elicitation potency relative to known allergens is of importance. Comparative human and / or animal test results are needed together with epidemiological knowledge for known allergens. For ethical reasons this information can not be obtained in humans for new proteins. However, for known allergens information can be obtained in allergic patients on threshold values, being the dose below which allergic individuals do not show objective clinical reactions upon exposure to the allergen.

Assessment of these threshold values is urgently needed and subject of current research. Also in validated predictive animal models, which as discussed above are not yet available, assessment of threshold doses for elicitation relative to those of known allergens will be possible. The threshold value for in example peanut, being the most potent allergen in elicitation reactions, can be used for a worse case approach. This means that the lowest level of peanut resulting in clinical reactions will be used as the highest exposure level of a protein of unknown allergenic potential.

Ideally, test systems for risk assessment should allow to calculate the probability that:

- a person becomes sensitised,
- and subsequently eats,
- such a combination and/or,
- such amounts,
- of food products containing the allergen under evaluation,
- at such levels,
- under such circumstances,
- that an adverse event should be expected to occur.

6.2 Outcome of discussions

In response to Mr. Houben's introduction the following remarks were made:

1. Allergenicity: Not all participants agreed on the statement that principally each protein has allergenic potential. It was argued that it is a matter of experience that in foods some proteins are allergenic, while others are not. Mr. Houben clarified that it is still not possible to exclude the possibility, though;
2. Risk assessment: The participants discussed whether or not to accept any hazard at all in view of allergenicity. Should a genetically modified food crop, which gives a positive result (i.e. an alert) in the decision tree, always be excluded? And how do we deal with possible false negative results? For a proper risk assessment further characterisation of the hazard and the risk is crucial. Important aspects to consider are: the allergenic potency (in sensitisation and elicitation phase), exposure and exposure conditions, prevalence and degree of sensitisation and incidence, nature and severity of effects;
3. Sensitisation: The participants agreed that protocols must be developed and validated for animal models to assess the sensitising potency of new food proteins relative to the existing allergenic food proteins or the development of other supplementary approaches. Together with the need of more epidemiological knowledge for known food allergens it was also agreed that protocols have to be developed for a more systematic approach to investigate (expected) exposure levels. Some items that were considered to play an important role in the exposure to food are:
 - ❑ (expected) consumption patterns, which determine the number of people, the frequency, and the doses of exposure;
 - ❑ inhalation of air borne food particles, or pollen of the genetically modified food crop may contribute to sensitisation;
 - ❑ compartmentalisation of the allergen (i.e.: is the protein in the part of the crop which we consume or inhale?);
 - ❑ processing of the food crop before consumption, and digestion upon consumption;
 - ❑ matrix of the food (e.g., it is hypothesised that the high fat content of peanut contributes to its allergenicity);
 - ❑ timing of exposure (e.g., infants younger than 1 or 2 years are easily sensitised);
 - ❑ outcrossing of the GMO-gene to other plant species, thereby possibly increasing exposure levels;

Scenarios for risk assessment

The following scheme summarises the discussion in this working session, showing possible scenarios for risk assessment, starting from two different results from hazard characterisation:

1. Scenario 1: The outcome of hazard characterisation is positive; the genetically modified food crop under evaluation contains a known allergen;
2. Scenario 2: The potential hazard of the genetically modified food crop can not be characterised because:
 - ❑ the source of the gene introduced into it has no history of use as a foodstuff

- or the GMO food contains newly engineered proteins.

Scenario 1: The outcome of hazard characterisation is positive; the genetically modified food crop under evaluation contains a known allergen

Risk assessment (Scenario 1):

1. Allergenicity:

Further characterisation of the hazard is needed, resulting in a ranked hazard in terms of:

- relative allergenic potency, determined with human sera and if possible by skin prick testing (SPT) and / or a double blind placebo controlled food challenge (DBPCFC). In addition, analyses of the physical-chemical properties of the food can be assessed.
- possible prevalence of sensitisation, estimated through epidemiological studies;
- possible altered exposure to the allergen as a result of the introduction of the GMO food crop, estimated by taking into account the above mentioned items;
- possible outcrossing of the transgene, and means to control this.

1. Exposure:

The safe level of exposure to the allergen must be determined and compared to the estimated exposure level after introduction/use of the GMO food crop. Possible results are:

- allergen exposure will appear above safe level. Introduction of GMO food crop is a high risk. No market introduction.
- allergen exposure will stay below safe level. In case it is also possible to show that the prevalence of sensitisation is low, the introduction of the food crop can be considered to be of low risk.

1. Experience of use:

The participants of the working session agreed that a general market introduction of a 'low risk' food crop should be preceded by:

- a limited market introduction (a restricted area/population, restricted exposure),
- labelling the products containing the GMO food,
- and monitoring possible adverse effects.

Evaluation of the data collected during a number of years must form the basis for deciding whether or not a general market introduction is acceptable.

Risk management (Scenario 1)

In case the evaluation shows that the GMO food crop can be used safely, a general market introduction must be accompanied by the following risk management measures:

- labelling products containing the genetically modified food crop, indicating the source of the transgene (e.g., 'contains apple derived protein'),
- continuation of the monitoring of possible adverse effects,
- defining the conditions of use (e.g. no use in infant formulas),
- and keeping control on exposure levels (no outcrossing, informing regulatory authorities about new applications of the genetically modified food crop).

Finally, the regulatory authorities must decide whether such measures of risk management are acceptable for admitting the GMO food crop on the market. This was not discussed during the working session.

Scenario 2: The potential hazard of the genetically modified food crop can not be characterised because:

- the source of the gene introduced into it has no history of use as a foodstuff**
- or the GMO food contains newly engineered proteins.**

Risk assessment (Scenario 2)

1. Allergenicity:

Insight into the relative allergenic potency:

- must be obtained through an animal model. However, such a model is not yet available, nor are there other tools for assessing the relative allergenic potency. In case good tools become available, the relative allergenic potency, together with the estimated exposure levels will indicate the relative risk.
- can not be obtained at present.
- Risk assessment must therefore start from the worst case scenario, i.e., the genetically modified food crop is as allergic as peanut.

1. Exposure:

At present, it is not possible to gain insight into the relative allergenic potency, and therefore the worst case scenario is also the starting point for establishing the safe exposure level of the GMO food crop. In case it can be shown that the estimated exposure level of the GMO food crop is below the safe exposure level of peanut (worst case), the GMO food crop may be considered as safe.

When in the future, the relative allergenicity of GMO food crops can be determined, and the safe exposure level can be established, it will be possible to calculate the relative risk.

The participants agreed that in both cases, outcrossing of the GMO-gene must be prevented in order to control the exposure to the GMO food crop.

2. Experience of use:

A limited market introduction and monitoring of possible adverse reactions prior to general market introduction, as described in scenario1 (c), would only be appropriate in case the relative risk can be established. At present, only the worst case scenario can be considered. If risk assessment shows that a given GMO food crop is safe, even in a worst case scenario, the product can be introduced on the market introduction without such limitations.

Risk management (Scenario 2)

The discussion during the working session on whether risk management is needed in case the GMO food crop is considered to be safe, was not concluded. Strictly speaking, only measures to control exposure to the GMO food crop are relevant. However, debate exists on whether also labelling of products containing GMO food crops and monitoring of effects after consumption are desirable.

In case it will be possible to calculate the relative risk, risk management measures as described in (1) are required.

7 Working Session 'Risk Communication'

Moderator: Dr. Edith Rameckers, EFA, NL
Introduction: Dr. Lynn Frewer, Institute of Food Research, UK
Report: Arie van Genderen and Huib de Vriend, Consumer and Biotechnology Foundation, NL

7.1 Introduction: Public perceptions of risk

What defines food choice?

In her opening remarks Ms. Frewer stressed the importance of the different factors likely to impact on food choices made by consumers. Three main factors were important in understanding food choice behaviours:

1. Individual consumer differences;
2. Cross cultural differences in preferences and values;
3. Globalisation of markets and the future of the food supply industrial strategies and regulatory issues and practices.

Many other aspects of food choice contribute to these three factors. Some examples of those aspects are the economics of food choice, food neophobia, (where people tend to avoid novel food products, or foods which they have not experienced before), private body consciousness, the role of values and beliefs, trust in government and risk perception and communication about food risks. The emphasis of the talk was on risk perception and communication, and public trust in information and regulatory practice.

Risk perception is socially constructed. Consumer behaviour is often driven by beliefs about risks, not by the technical risk estimates provided by scientists or technical experts. People tend to feel more threatened by risks which they perceive to be involuntary, unnatural, and uncontrolled (by science or themselves). For example, people accept the risks of driving a car because they perceive that they choose to engage in the risky activity, and there is a high personal benefit attached to the risky activity. Public reaction against nuclear power was driven by perceptions that the risks of the technology were imposed upon the public, were unnatural, and benefited industry, not people in general.

Communication

Early research into public beliefs about genetic modification of foodstuffs was driven by some erroneous assumptions. Firstly, it was believed that the general public would view genetic modification as a unitary technology, and people did not differentiate between different applications. Secondly, the public could be educated to accept the technology, and represent the science in the same way that experts did. Aligning public views with those of experts through educational processes would result in public acceptance of genetic modification. Social context and individual differences in attitudes were not considered important. Today, it is clear that the matter is much more complicated. Consumer attitudes are linked to a range of values and beliefs, health concerns, attitudes towards the environment, and trust in government and regulatory practices. Factors effecting food choice are complex, and cannot be represented in a simple way.

Traditionally, new products have entered the market place as a result of "science push" (developing novel foods because it is technically possible). There is now more emphasis on "market pull". This implies the need for better anticipation of what consumers want, rather than trying to convince them that new products are acceptable. Distrust in science and government is fuelled by the belief that the real risks are being hidden to promote vested interests.

Risk perception, trust and information provision

Trust in information sources is likely to be influential in determining how people react to risk communication. Differences in the way source characteristics are perceived by the public have been observed, although these differences are prone to individual differences and cross-cultural variation. In the UK government and industry are distrusted, pressure groups and medical sources highly trusted. Information sources perceived to be highly competent, but are simultaneously distrusted, may be perceived as distorting information to protect their own interests - that is, the public will not attend the information as they perceive it is prone to reporting bias.

Consumers are likely to ask two key questions about genetically modified foods:

1. What are the benefits: new technologies are only developed if someone benefits from it. In the case of genetic modification there seems to be little or no benefit for the consumer. So people ask, why develop a technology if there is no real benefit to the consumer, but only to industry? This is particularly important if people perceive that consumers or the environment are accruing the risks, but industry is accruing profits or other advantages.
2. What are the risks? If there are risks and no visible benefits, why develop the technology in the first place?

Cross cultural differences in the importance of risk perceptions in food choices have been identified. For example, North Europeans tend to make decisions based on "risk aversion", while Southerner Europeans are more likely to make decisions based on acquiring benefit. In addition, Southern Europeans are more concerned about the potentially negative effects of genetically modified foods on quality. Other differences in perceptions are linked to gender, ethnicity and social class. Those who perceive that they are socially excluded from risk management processes (poorer people, women, members of particular ethnic groups) are more likely to perceive technologies to be high in risk, whilst simultaneously expressing the need for greater public involvement in risk management processes.

New risk management strategies

Ms. Frewer concluded her contribution by stressing the need to develop new risk management strategies. The "ownership" of risk regulation and scientific development should be brought more into the public domain through increased regulatory and scientific transparency, and proactive forms of communication. In general, people tend to prefer decision making under conditions of certainty. People are not necessarily "risk" adverse, but they are "loss" adverse. They are willing to take risks when they think it appropriate (for example, they are more willing to accept genetically modified foods if there is a clear and tangible consumer benefit). It is important to develop meaningful ways to communicate about the different dimensions of scientific uncertainty.

7.2 Outcome of discussions

Consumers and patients

Following Ms. Frewer's introduction, the need to make a distinction between the general consumer and consumers with an allergenicity to certain foodstuffs was identified. In general, the perception of risks is linked to how people deal with uncertainties about different hazards in their ordinary lives. Allergy patients are already aware of specific risks, and are more alert to the possibility of the hazard affecting them. The information needs of patients are very different to the public in general. Therefore it was concluded that a distinction between patients and the general consumer should be made when considering the development and provision of risk information.

GMO foods and traditional foods

One of the participants put forward the question whether a distinction should be made between genetically modified novel foods and traditional foods. After some discussion it was agreed that traditional ingredients, which might be problematic to patients with specific allergies, might enter the food chain in unexpected products as a result of more centralised production methods.

Public participation & communication

In 1995 FAO and WHO set up a risk management scheme, emphasising the relation between risk assessment, risk management, public perception and risk communication⁴. According to several participants, this scheme reflects a very top-down approach. Public participation should be incorporated in this. There should be equal representation in the debate about food risk between consumers, industry, trade and government representatives.

In this regard, some concerns about the representativeness of delegates to consultation exercises were expressed. Lack of interest of the public in decision processes may be explained by a lack of transparency and insufficient level of true representation in these processes. Too often, communication is limited to the level of NGO's and the public is not directly involved.

The language used by the public, scientists, industry and government may be very different. For these reasons, risk communication should be improved by starting to develop a common language, that can be understood by all actors and stakeholders in the debate about risk. This debate must also address the issue of benefits resulting from the application and development of the technology. Regional and demographic differences in the EU concerning trust in regulatory systems and food culture should be taken into account.

Information strategies

1. *Dual information strategy*: It became clear that there is a need for a dual strategy on issuing the proper information regarding genetically modified foods. General information should be available for the general public, whereas patients and other special interest groups should be addressed with

⁴ FAO/WHO (1995) Consultation on Risk Analysis: Report. FAO/WHO, Geneva

specific information tailored to their needs. That is, the general information provided to all consumers should be supplemented with specific and more detailed information for the target patient group. Some people felt that genetically modified foods should be identified as a separate issue in the information provided.

2. *Information design:* There was a lot of discussion regarding which issues the information should address. Consensus was, however, reached regarding inherently risky products: they should not be allowed on the market in the first place. Information for the general consumer should be (scientifically) correct, comprehensible and include communication about both the composition of the product as well as the production system used in its manufacture. Information for patients should be the same, with some additional information on specific compounds found in particular products that might pose a hazard to specific patient groups. Science should provide the necessary data in an unbiased way and also give information on possible benefits of novel products or ingredients.
3. *Access to information:* Representatives of trade and industry were of opinion that information on the production system should be available on special request only. In their view, genetic modification is nothing more than a production tool. Representatives from patient and consumer organisations disagreed on this point and stressed the importance of freedom of choice. In other words, the freedom to choose GMO-free foods.
4. *Presentation:* The presentation of the information should be through different information channels. The label is the most important source of information about specific products, particularly as this information is available at "point of sale". The Nordic countries are in favour of a special mark on the label of the product to indicate changes in recipe as a warning to patients and general consumers. Other information sources, such as brochures, the Internet, and articles in magazines and newspapers should deal with more general aspects of products, production system and possible allergenic compounds. An up to date databank is essential if information is to be accurate and based on the most appropriate and up to date scientific information available.
5. *Tone:* The 'tone' of the information has to be factual /honest, realistic and the information given should also mention and explain the uncertainties associated with risk assessment practice. It should not give rise to unnecessary alarm, but on the other hand it should not be so reassuring that people become careless about food choice decisions. It should be made clear that a 100% safety does not exist, and that people at risk should still be cautious in making food choice decisions.
6. *Labelling:* Labelling is seen as the prime source for informing consumers and patients about new allergenic properties in food. All other information sources (databases, brochures, articles, internet) should be used as well, in particular databases. This database should be an easy accessible and European wide.
The trade representative in the group (again) stressed the point that no link should be made between GMOs and allergenicity. The large majority of participants felt that products with new allergenic properties should not be allowed on the market. After some discussion it was agreed that consumers should be free to choose between GMO-containing and non-GMO products, on basis of good information and clear labelling.

7. *Source of information:* The issue of who should be the source of which information was addressed only briefly. Some participants referred to the Eurobarometer on Biotechnology. It was stressed that the public has very mixed views on the reliability of different information sources. Several participants stressed the importance of "educating" journalists on the various aspects of food allergenicity. Special attention should be given to the use of reliable information sources.

Conclusions from Working Session 2

In the discussion of this working session, following conclusions were made:

1. Information strategies should be developed at a European level. Representatives of consumers and patients organisations should properly be represented in the decision making process. It might be helpful to start a European Platform for the development of information strategies, a platform that gathers regularly to keep all parties updated. One of the first tasks of the platform would be to make an inventory on the state of the art in the various member states regarding information provision about food allergenicity.
2. Effective risk communication should start with developing a language that can be understood by lay people.
3. Labelling is seen as the prime source for informing consumers and patients about new allergenic properties in food. All additional appropriate information channels should be used to deliver the information to target audiences. These include brochures, the Internet, the proposed European database and articles in the relevant magazines and newspapers. It might be useful to educate journalists on the matter of allergenicity by leading them to reliable information sources.
4. Information should be given in an unbiased way which is understandable to the general public. Uncertainties inherent in risk management should be explained. All peoples concerns should be discussed, even if they are unsubstantiated by scientific evidence. Patients need additional information, geared towards their specific problem. Information from various sources should be consistent.
5. In developing information strategies cultural and social differences should be taken into account. These differences might embrace the way in which different consumers use and treat food, and also the way in which they emphasise food related risks.
6. The representatives of the consumer and patient organisations were very clear about their preferred strategy for the market release of genetically modified products. These products should *always* be labelled as such and new genetically modified products that might contain known allergens should not be allowed on the market. Some industry and trade representatives disagreed on this point. A final conclusion was that there needs to be further debate about the differences between selective breeding and genetic modification.

Annex 1: Workshop programme

Genetically modified foods and allergenicity: safety aspects and consumer information

Workshop, 28-29 May 1999, Breukelen, The Netherlands

Day 1, Friday, May 28

- 13.00 – 13.30 *Registration*
- 13.30 Opening by chairman
Dr. Carsten Bindslev-Jensen, Odense University Hospital, DK
- 13.45 What genetically modified foods can we expect? How do we assess their safety?
Dr. Harry Kuiper, State Institute for Quality Control of Agricultural Products (RIKILT) NL
- 14.15 *Break*
- 14.45 Clinical aspects of food allergy
Dr. Carsten Bindslev-Jensen, Odense University Hospital, DK
- 15.15 IFBC/ILSI decision tree for the assessment of the allergenic potential of GMO-foods
Prof. dr. Steve Taylor, University of Nebraska, US
- Frans van Dam**, short introduction to the Working Sessions
- 15.45 *Break*
- 16.15 – 18.30 Parallel Working Sessions (to be continued on May 29, in the morning)
- 1a. Hazard assessment
 moderator: **Prof. dr. Paul Davis**, Unilever, UK
 introduction: **Dr. Wolf-Meinhard Becker**, Borstel Res. Center, GE
- 1b. Risk assessment
 moderator: **Dr. André Penninks**, TNO-Voeding, NL
 introduction: **Dr. Geert Houben**, TNO-Voeding, NL
2. Risk communication
 moderator: **Dr. Edith Rameckers**, EFA, NL
 introduction: **Dr. Lynn Frewer**, Institute of Food Research, UK
- 19.00 Dinner

Day 2, Saturday, May 29

8.30	Working sessions – continued
11.00	Break
11.30 – 13.00	Plenary session – report from working sessions by moderators
13.00 – 14.30	<i>Lunch</i>

Annex 2: List of participants

Dr. Marcos Alcocer	Institute of Food Research	UK
Dr. Steven B. Andersen	Royal Veterinary and Agricultural University, Dept. of Agricultural Science	Denmark
Ms. Birgit Beck	Verein für Konsumenteninformation	Austria
Dr. Wolf-Meinhard Becker	Borstel Research Center	Germany
Dr. Carsten Bindslev-Jensen	Odense University Hospital, Department of Dermatology	Denmark
Ms. Ulla Bindslev-Jensen	Odense University hospital, Hospitalsapoteket FAC	Denmark
Ms. dr. S. Margreet Bloemers	Consumer & Biotechnology Fnd.; Nederlandse Voedselallergie Stichting	Netherlands
Dr. Andrew Chesson	Rowett Research Institute	UK
Ms. Erika Colen	Astmafonds	Belgium
Mr. Frans W. van Dam	Consumer & Biotechnology Fnd.	Netherlands
Prof. Paul J. Davis	Unilever	UK
Ms. Karen I. van Drongelen	Voedingscentrum	Netherlands
Mr. Ernst-Michael Epstein	Arbeitsgemeinschaft für Verbraucherverbände	Germany
Ms. dr. Lynn Frewer	Institute of Food Research	UK
Mr. Arie W. van Genderen	Consumer & Biotechnology Fnd.	Netherlands
Dr. Cor Glas	Friesland Nutrition	Netherlands
Dr. Richard E. Goodman	Monsanto	USA
Ms. Karin Groothuis	Consumentenbond	Netherlands
Mr. J.A. Haagsman	Edah	Netherlands
Ms. Betina Hjorth	Astma-Allergiforbundet	Denmark
Ms. dr. Elisabeth Hogendoorn	Novartis Seeds BV	Netherlands
Dr. Geert F. Houben	TNO-Voeding	Netherlands
Ms. Marianne Jarl	Astma och Allergi Förbundet	Sweden
Ms. dr. Merja Kajosaari	Allergia – ja Astmaliitto ry	Finland
Mr. Jan-Willem van der Kamp	TNO-Voeding	Netherlands
Ms. Beate Kettlitz	BEUC	Belgium
Ms. dr. Ariane König	Monsanto	Belgium
Dr. Harry A. Kuiper	State Institute for Quality Control of Agricultural Products	Netherlands
Dr. Martinus Løvik	National Institute of Public Health	Norway
Ms. Elvira L.J.M. Luykx	Koninklijke Numico NV	Netherlands

Mr. Jan A.G. Madlener	Novartis Seeds BV	Netherlands
Ms. dr. Charlotte Madsen	Danish Food Administration	Denmark
Ms. Leena Mannonen	National Food Administration	Finland
Prof.dr. K. Nékám	Hungarian Society of Allergology and Clinical immunology	Hungary
Mr. Louis van Nieuwland	Consumentenbond	Netherlands
Ms. dr. Annette Olesen	Royal Veterinary and Agricultural University, Dept. of Agricultural Science	Denmark
Dr. André H. Penninks	TNO-Voeding	Netherlands
Dr. Lars K. Poulsen	Laboratory of Medical Allergology Rigshospitalet	Denmark
Ms. dr. Edith M.A.L. Rameckers	European Federation of Asthma and Allergy Associations	Netherlands
Dr. Ronald van Ree	Centraal Laboratorium voor de Bloedtransfusie	Netherlands
Ms. dr. Karin Retzlaff	BEUC	Belgium
Ms. Marianne Schauzu	Bundesinstitut für Gesundheitlichen Verbraucherschutz und Veterinärmedizin	Germany
Mr. J. Piet M. Schenkelaars	Schenkelaars Biotechnology Consultancy	Netherlands
Dr. Benoit Schilter	Nestec Ltd. Research Center	Switzerland
Ms. Stine Sem	Norwegian Consumer Council	Norway
Dr. Maurice R. Smith	Unilever / ILSI	Netherlands
Prof.dr. Steve L. Taylor	University of Nebraska, Dept. of Food Science & Technology	US
Ms. Erja Tommila	Allergia – ja Astmaliitto ry	Finland
Mr. Rob Top	Ministry of Health	Netherlands
Dr. Bert A. Uijtewaal	AgrEvo	Belgium
Ms. Lorena Valdicelli	Comitato Consumatori Altroconsumo	Italy
Mr. Huib C. de Vriend	Consumer & Biotechnology Fnd.	Netherlands
Ms. dr. Barbara Weber	Oko-Institut	Germany
Ms. Therese Wegmuller	Das BAND	Switzerland
Ms. dr. Janine van der Wiel	Gezondheidsraad	Netherlands