

TNO International Food Allergy Forum

15-16 April 2002

Abstracts of Lectures and Posters

Golden Tulip Conference
Hotel Leeuwenhorst
Noordwijkerhout
the Netherlands



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TNO International Food Allergy Forum

**15 - 16 April 2002
Noordwijkerhout, the Netherlands**

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Key to the abstracts of lectures and posters

Abstracts of lectures and posters are grouped separately.

The lectures are grouped according to the daily programme.

The posters are grouped (i) according to food allergens and (ii) in an alphabetical order according to the first author.

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Welcome at the TNO International Food Allergy Forum!

Dear participant,

We are pleased to present you the lecture and poster abstracts of the TNO International Food Allergy Forum.

Food allergy is a topic of growing concern. Not only traditional food products, but also novel foods and food ingredients may elicit allergic reactions, causing a wide range of symptoms in sensitive subjects. Recognising the importance to control allergic reactions to foods, TNO Nutrition and Food Research chose food allergy as the subject of its conference, the TNO International Food Allergy Forum.

The conference's main objectives are:

- to provide a unique platform for the food industry, science, regulatory authorities and the consumer;
- to exchange information and experiences on the various aspects of food allergy;
- to review current knowledge related to food allergy;
- to discuss strategies for the control of allergic reactions to food.

You are cordially invited to take part in the discussions with participants from different disciplines and to meet business relations in your area. We wish you an active and fruitful meeting!

Kind regards,

André Penninks
Chairman of the Organising Committee

PROGRAMME

Monday, 15 April 2002

09.15 *Opening of the TNO International Food Allergy Forum*
A. Penninks
TNO Nutrition and Food Research, the Netherlands

Plenary meeting

The food allergy area

Chair: A. Penninks
TNO Nutrition and Food Research, the Netherlands

09.30 *Introduction by the chair*

09.45 *Food allergy issues from an industry's point of view*
C. Hischenhuber
Nestlé, Switzerland

10.15 *Food allergy: mechanisms and clinical aspects*
C. Bindslev-Jensen
Odense University Hospital, Denmark

10.45 Coffee/tea break

11.15 *Food allergy: an increasing concern?*
M. Jansen
TNO Nutrition and Food Research, the Netherlands

11.45 *Food allergy and product development*
N. Craddock
Nestlé, UK

12.15 *Product stewardship: where are we now in protecting the allergic consumers?*
R. Crevel
Unilever, UK

12.45 Lunch break

Monday, 15 April 2002

Parallel session 1

Product development - general safety assessment

Chair: G. Houben
TNO Nutrition and Food Research, the Netherlands

14.00 *Introduction by the chair*

14.15 *Identification of potential allergenic hazards in food product development*
S. Taylor
University of Nebraska, USA

14.45 *Current practice in risk assessment: protection of all consumers!*
C. Madsen
Danish Veterinary and Food Administration, Denmark

15.15 Coffee/tea break

15.45 *Internal and external factors and criteria in decision-making*
J. Bindels
Numico, the Netherlands

16.15 *Regulatory approval: do we accept new cases of food allergy?*
R. Top
Ministry of Health, Welfare and Sport, the Netherlands

16.45 End of parallel session 1

Monday, 15 April 2002

Parallel session 2

Efficacy and safety evaluation

Chair: R. Crevel
Unilever, UK

14.00 *Introduction by the chair*

14.15 *Development and testing of hypoallergenic formulas*
C. Cordle
Ross Products, USA

14.45 *Possibilities and limitations of agricultural GM crop-products*
R. Goodman
Monsanto, USA

15.15 Coffee/tea break

15.45 *The role of intestinal flora in food allergy*
B. Björkstén
Karolinska Institute, Sweden

16.15 *Can specialty products prevent food allergy?*
L. Knippels
TNO Nutrition and Food Research, the Netherlands

16.45 End of parallel session 2

Monday, 15 April 2002

Interactive round-table discussion 1

Risk evaluation of novel proteins

Taking into account that every foreign protein may act as an allergen, will we be able to define an acceptable risk level? Will we be able to assess new proteins on criteria derived on the basis of this risk level?

Chair: S. Taylor, University of Nebraska, USA
G. Houben, TNO Nutrition and Food Research, the Netherlands

17.00 Chair's introduction

17.15 Discussion

18.30 End of round-table discussion

Interactive round-table discussion 2

Registration and post-launch monitoring

Registration of food allergic reactions and post-launch monitoring may be used as tools in food safety monitoring. Will this be feasible and reliable?

Chair: M. Løvik, National Institute of Public Health, Norway
M. van Dusseldorp, TNO Nutrition and Food Research, the Netherlands

17.00 Chair's introduction

17.15 Discussion

18.30 End of round-table discussion

Interactive round-table discussion 3

Labelling of food allergens

Can (defensive) labelling act as a substitute for GMP?

Chair: D. Reading, The Anaphylaxis Campaign, UK
N. Craddock, Nestlé, UK

17.00 Chair's introduction

17.15 Discussion

18.30 End of round-table discussion

20.30 Conference dinner

Tuesday 16 April 2002

Parallel session 3

Product stewardship - risk assessment

Chair: T. Brussaard
TNO Nutrition and Food Research, the Netherlands

09.15 *Introduction by the chair*

09.30 *Threshold levels for challenge reactions: how much is too much?*
C. Bruijnzeel-Koomen
University Medical Centre/Utrecht University, the Netherlands

10.00 *Detection of food allergens: how low can we go?*
S. Koppelman
TNO Nutrition and Food Research, the Netherlands

10.30 Coffee/tea break

11.00 *Exposure assessment: do we have any idea on intake?*
J. Hourihane
Southampton General Hospital, UK

11.30 *Changes in practice of risk assessment: from possibility to probability*
G. Houben
TNO Nutrition and Food Research, the Netherlands

12.00 End of parallel session 3

Tuesday 16 April 2002

Parallel session 4

Product stewardship – risk management tools

Chair: S. Taylor
University of Nebraska, USA

09.15 *Introduction by the chair*

09.30 *HACCP to control allergens in food manufacturing processes*
D. Cromie
Unilever Bestfoods, UK

10.00 *Allergens: why do we need to label?*
N. Craddock
Nestlé, UK

10.30 Coffee/tea break

11.00 *The importance of a food product data base on allergens for consumers and industry*
M. van Dusseldorp
TNO Nutrition and Food Research, the Netherlands

11.30 *Do quality systems guarantee safe food for allergic persons?*
H. Byrnes, CIES, France

12.00 End of parallel session 4

Tuesday 16 April 2002

Final plenary meeting

Food allergy - the way ahead of us

Chair: T. Brussaard
TNO Nutrition and Food Research, the Netherlands

- 13.30 *Chair's summation of round-table discussion 1*
S. Taylor, University of Nebraska, USA
G. Houben, TNO Nutrition and Food Research, the Netherlands
- 13.45 *Chair's summation of round-table discussion 2*
M. Løvik, National Institute of Public Health, Norway
M. van Dusseldorp, TNO Nutrition and Food Research, the Netherlands
- 14.00 *Chair's summation of round-table discussion 3*
D. Reading, Anaphylaxis Campaign, UK
N. Craddock, Nestlé, UK
- 14.15 *Increasing awareness of food allergy*
F. Timmermans
European Federation of Asthma and Allergy Associations (EFA), the Netherlands
- 14.45 *Looking into the future: scientific and regulatory aspects*
K. Vierk and K. Falci
Food and Drug Administration, USA
- 15.30 *Closing the TNO International Food Allergy Forum*
A. Penninks, TNO Nutrition and Food Research, the Netherlands
- 15.45 End of conference

LECTURES

Food allergy issues from an industry's point of view

C. Hischenhuber

Nestlé, Switzerland

Food allergy is recognised as a significant public health problem. The prevention of severe allergic reactions to food can only be achieved by interdisciplinary collaboration among allergologists, food technologists, chemists, food industry and regulatory bodies. In order to be able to avoid his/her specific food allergen, the allergic consumer needs sufficient and correct information on the nature and composition of each product. Prevention of incidents is therefore a combined action of consumers and producers: the food allergic consumer must be able to make an informed choice and the producer must provide him with the information to enable him to make this choice.

The food manufacturer can deal with this issue by having implemented an allergen management policy, i.e. by having implemented appropriate GMP (Good Manufacturing Practices), having fully integrated allergen hazards in the HACCP (Hazard Analysis of Critical Control Points) plan and finally by accurate labelling of products. However, allergen management is not an easy task for the food industry. Already during the development of new products all parties involved need to consider allergen issues. This encompasses the input of a wide spectrum of experts such as engineers, technologists, hygienists, quality assurance managers, regulatory affairs specialists, marketing, etc. Accordingly, allergen issues need to be included in employee training and commitment of the whole factory personnel is required.

When looking at possible allergen hazards, automatically the crucial question arises: how much is too much? The amounts of allergen required to elicit a reaction are thought to differ considerably from one allergic individual to another and are not yet well defined. Better knowledge of these thresholds is a prerequisite for risk assessment of food allergens. When assessing the risk of exposure to a chemical, several well-defined steps are involved: hazard identification, hazard characterisation, exposure assessment and calculation of probability (frequency) of the adverse event (risk characterisation). When making risk assessment of food allergens their unique features as compared to chemicals or pathogens need to be considered. Besides the establishment of thresholds for food allergens, more knowledge of the prevalence of food allergies needs to be gathered. Another difficult point will be the establishment of safety factors or margins bearing in mind how threshold studies are performed (exclusion of the most sensitive individuals).

The possibility of introducing unlabelled allergens into a food may arise from various sources, e.g. from raw materials that contain 'hidden' allergens. For this reason, not only the manufacturer of the finished food products, but also the respective raw material suppliers must have an allergen management policy in place. Close collaboration of purchasing and quality assurance departments with the suppliers is therefore of paramount importance. Other risks that have to be minimised are shared storage places, shared equipment, and shared production lines. It must be emphasised that it is definitely not always feasible to have

a separate production line for each recipe. However, in certain cases effective cleaning procedures are possible when switching from a product that contains a critical allergen to a product that does not contain that allergen. Production scheduling by grouping the production of similar products has to be defined. The cleaning procedure itself must be described, implemented and validated by visual inspection and analytical tests. The cleaning efficiency is then defined as a CCP (critical control point). In other cases, e.g. the production of chocolate confectionery, it is not feasible to clean shared lines to an extent that would allow precautionary labelling of allergen traces to be avoided. A risk of introducing 'hidden' allergens into products that must not be overlooked is rework. Correct labelling of containers containing rework as well as strict adherence to the policy like-into-like (e.g. egg pasta rework is only introduced into egg containing pasta) has to be under control.

To check whether any unlabelled 'hidden' allergens are present in raw materials or food products, or for checking cleaning effectiveness after product switchover, analytical methods for the detection and/or semi-quantitative determination of critical allergens are useful tools (ELISA, Western blot, immune-electrophoresis, PCR of DNA used as marker for the presence of an allergen). However, several points must be critically considered when developing, validating and applying analytical methods for allergen detection including:

- is the detection limit of the method relevant?
- is the analytical parameter measured relevant?
- is the method appropriate for the food matrix to be analysed?
- and, last but not least, does the analytical result give useful information on the safety of the product for the allergic consumer?

For example, analysis of a finished product sample may even be counterproductive if visual inspection of the production line and common sense suggests that efficient cleaning is not possible (heterogeneous distribution of cross-contact, e.g. nut pieces).

It must be emphasised that precautionary labelling ('may contain traces of milk', etc.) should not be done to replace GMP, but is, in certain cases, the only means to produce a wide variety of products and, at the same time, to protect allergic consumers. The presence of major allergens should always be labelled regardless whether they are part of a compound ingredient, an additive or a major ingredient. On the other hand, future labelling regulations should take into account scientific progress on allergenicity assessment of specific ingredients derived from food containing critical allergens such as fish gelatin, refined soybean oil, wheat maltodextrin etc., thus avoiding eventual overdoing allergen labelling which would create more confusion than guidance for the allergic consumer.

References

1. Huggett, A.C. and Hitchenhuber, C., 1998. Food manufacturing initiatives to protect the allergic consumer. *Allergy* 53 (Suppl. 46): 89-92.
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3. Hourihane, J.O'B., Kilburn, S.A. and Nordlee, J.A. et al., 1997. An evaluation of the sensitivity of subjects with peanut allergy to very low doses of peanut: a randomised DBPCFC study. *J. Allergy Clin. Immunol.* 100: 596-600.
4. Crevel, R.W.R., 2001. Risk assessment for food allergy - the industry viewpoint. *Allergy* 56 (Suppl. 67): 94-97.

Food allergy: an increasing concern?

M. Jansen

TNO Nutrition and Food Research, the Netherlands

Food allergies receive more and more attention by researchers, food industry, medical doctors, legislative bodies, and last but not least consumers. However, has the prevalence of food allergies indeed increased?

A literature search showed that data on the prevalence of food allergies in the general population in Europe are scarce. Several reasons underlie this observation. First, the diagnosis of food allergy is difficult to assess based on questionnaires. Second, most studies were performed in patient populations, which makes it difficult to draw conclusions about the prevalence in the population at large. Third, comparability between studies was limited due to differences in methods of measuring food allergies and used study designs. The big issue is that prevalence of perceived food allergies is high in Europe (from 4 to 27%), while the prevalence of proven food allergies is much lower, i.e. from 1 to 7%. Foods or food ingredients alleged to cause allergy were largely the same in the different countries, although their relative importance varied per country. The major allergenic foods are milk/dairy products, peanuts, soy, (tree)nuts, and cereals containing gluten, fish, crustaceans, and eggs. Other foods or food ingredients mentioned to cause allergic reactions by consumers are fruits, vegetables, chocolate, meat, sugar and sweets, soft drinks, alcoholic beverages, herbs and condiments and food additives.

Based on the published data it is not possible to answer the question whether the prevalence of food allergy is increasing. However, there are indirect indications that suggest an increasing prevalence of food allergies. First, food allergy often goes together with other allergies, like asthma and hay fever. It is indicated that such other allergies are increasing. This may imply that the prevalence of food allergy is increasing too. Second, a study on children allergic to peanuts inquired the prevalence of peanut allergy among their siblings, parents and grandparents. Peanut allergy occurred most in the youngest generation and least in the oldest generation. Although this finding indicates a rise in food allergy, the other side of the story is that also the consumption of peanuts was higher in the younger generations. Third, it is thought that the prevalence of food allergy rises because the variety of (processed) foods people consume has never been greater before.

The challenges for the future are at the one hand to perform epidemiological studies that better assess prevalence of food allergies in the general population, and at the other hand to provide consumers with better information about the foods they purchase and specifically about their composition.

Food allergy and product development

T. Hatzold

Kraft Foods, Germany

Product development includes a variety of different targets such as development of new products, improvement of the quality of existing products, and improvement of the cost-effectiveness of the processes. The consumer is the main focus and the product developer's aim is to fulfil his or her needs and desires as much as possible. However, there are items setting boundaries to the flexibility and these are product safety, legality, company internal guidelines, and manufacturability.

Safety

Safety of the product is an absolute must, and this is defined in the EU regulation on general principles and requirements of food law. The regulation stipulates that in determining whether a product is safe regard shall be had "to the information provided to the consumer, including information on the label, or other information generally available to the consumer concerning the avoidance of specific adverse health effects from a particular food or category of foods". This shows product safety goes beyond composition and physical properties of a product and may also cover labelling.

Legality

Legality is another pre-requisite and there are indeed a huge number of different regulations to be followed especially when products are sold across the world. Relevant in this context are initiatives of regulators to lay down rules for allergen labelling, which are being developed/updated within Codex Alimentarius, in the USA and the EU. Common criteria for inclusion of foodstuffs into the list of allergens are essential, so that world-wide harmonised lists of allergens can be established. Otherwise, it would be too complicated a task to provide for adequate control of presence of allergens in raw materials, throughout the food chain.

Internal guidelines

To fulfil safety and legal requirements, and consumer expectations with regard to quality, food companies usually establish internal procedures, guidelines, and policies, which have to be followed in product development across all the countries where the company is active. More and more food companies nowadays include allergen control procedures within these guidelines, which should cover:

- Product design to avoid presence of allergens where appropriate
Avoidance of allergens is not always easy given that some popular ingredients such as milk and eggs are most common allergens. For example, it is neither possible nor desirable to manufacture a milk chocolate or a cheese without milk. However, where there is a choice, an allergen should be avoided. Use of potato starch instead of wheat starch is an example to avoid the allergen wheat. Another example is the use of rice crispies instead of wheat crispies.
- Specifications of raw materials and control of suppliers
Detailed raw material specifications and questionnaires to the suppliers need to be developed in order to ensure that all allergens contained in a raw material are made

known to the food manufacturer. When new suppliers are identified, they should be evaluated and approved by the manufacturer to assure that incoming products fulfil all requirements, especially regarding presence of allergens.

- **Manufacturing controls to exclude presence of unintentional allergens**
To exclude presence of unintentional allergens in a product, tight controls in the manufacturing facilities are required, especially to avoid any unintentional carry-over of an allergen into another product. When introducing a new potentially allergenic ingredient, special care has to be taken to avoid any implications for other products manufactured in the same factory. In certain cases it may even be necessary to abandon the idea of introducing a new product variety with an allergenic ingredient, in order to avoid carry-over into the other products manufactured in the same facility.
- **Labelling**
Elaboration of appropriate labelling is an essential part of product development and it must be made clear to the consumer, which allergen is present in a product. It is also important to choose the right term in the languages of all the countries where the product is sold, to assure clear information to the consumer.
- **World-wide update of scientific and legal developments**
It is important to maintain and update an internal list of allergens that should take into account all allergen issues world-wide and consequently may be broader than 'legal' lists existing for specific countries. This list should also contain exemptions for those ingredients that are purified from proteins and thus are not considered allergens (e.g. purified wheat starch hydrolysates, refined oils).

Innovative products

Development of products beyond 'conventional' product development is a separate issue, and the following areas are of interest:

- products designed to have reduced allergenicity;
- products consisting of or containing novel foods/ingredients, containing new proteins that need assessment of allergenic potential; and
- Products based or derived from GMOs (genetically modified organisms) containing new proteins that need assessment of allergenic potential.

Examples for products with reduced allergenic potential are hypoallergenic infant formulae, but discussions of details in the area of dietetic food goes beyond the scope of this paper. Development of foods intended for general food consumption but with reduced allergenicity is another challenging task for the future.

In the area of novel foods, more and more consumer demand for exotic fruits and other innovative products is seen. Exotic fruits are not per se more allergenic than conventional ones, but careful evaluation of all available scientific data and application of safety evaluations according to novel foods regulations are necessary when introducing novel products.

For the assessment of the allergenic potential of foods derived from GMOs it is important for the product developer to follow internationally agreed guidelines (e.g. FAO/WHO 2001, Evaluation of Allergenicity of Genetically modified food) and of course all legal requirements in all countries where the product is to be marketed. Special care has to be taken to assure that no accidental co-mingling with material derived from GMOs occurs, which have not been assessed for allergenicity and therefore are not approved for human consumption.

All in all, the product developer in food industry can play a significant part in supporting the allergy sufferers in dietary management of the disease.

Product stewardship: where are we now in protecting the allergic consumers?

R. Crevel

Unilever, UK

Product stewardship is a principle that directs all actors involved in the life cycle of a product to take responsibility for the impacts to human health and the natural environment that result from the production, use and disposal of the product. Much of the initial and, to some extent continuing, focus of product stewardship has been on the environmental impact of products. However, as the definition stresses, impact on human health is an equally important element. Food allergy is a condition, which has generated growing interest over the last decade, in part because of the possible link to the rising incidence of atopic diseases, but also through a number of well-publicised incidents. This increasing interest has led to action by food manufacturers, retailers and regulatory bodies, both national and international, to ensure that the risk to food allergic individuals is minimised. Food allergens principally affect consumers at the time they buy and consume the product, although they also present a more limited risk to workers handling the materials. However, in order to protect the health of the consumer, the handling of food allergens must be considered at all points in the supply chain. Manufacturers have a key role to play in this process. To protect existing allergic consumers they need to inform them and/or, where appropriate, prevent inadvertent contact with specific allergens, e.g. through cross-contact. To protect susceptible consumers from allergy to novel proteins, they must be able to form a judgement on the potential allergenicity of these proteins. Manufacturers have developed systems to address these issues. These systems usually start with the development of policies, which provide a framework within which specific activities occur. These activities include, for example, design of facilities to prevent cross-contact, introduction of HACCP systems, supplier audits and monitoring systems.

This paper will discuss what has been achieved to date, illustrating with examples from the Unilever experience in this field. Activities to protect the allergic consumer are, of course, limited by available knowledge as well as by inadequate dissemination of existing experience. The paper will highlight those gaps and outline current work to address these shortcomings.

Identification of potential allergenic hazards In food product development

S.L. Taylor.

University of Nebraska, USA

In the development of new food products, the potential allergenicity of the food and its various components including novel proteins and their source materials must be taken into consideration. As new food products are developed, potential allergenic hazards can arise in several different ways with varying degrees of risk. First and foremost, the new food product might contain known allergenic foods or components derived from such sources. Second, an entirely new food may be introduced into a region, country or locale. This food may be of unknown allergenicity in the new area but could be a product that is routinely consumed in other parts of the world. The third possibility involves the introduction of novel food components containing novel proteins not previously included in human diets (e.g. cottonseed meal/protein) or involves a possible increase in exposure to the particular novel protein (e.g. the approval of lysozyme as a food additive). The fourth possibility involves the introduction of a genetically modified food into the diet that contains one or more novel proteins. While this final possibility is likely to be the least risky (at least with respect to the current generation of genetically modified foods), it seems to garner the most attention and concern.

Introduction of known allergenic foods or food components

The inadvertent ingestion of allergenic foods is potentially hazardous for perhaps 2-2.5% of consumers in North America and Europe. The potential hazard is greatest for infants and young children where the prevalence of food allergy is in the range of 5-8% of the population below the age of 3 years. In the U.S. alone, 6-7 million Americans are estimated to have food allergies. Thus, the introduction of known allergenic foods or food components during product development carries acknowledged risks for consumers with existing food allergies. While many foods are known to be allergenic, 8 foods or food groups (milk, eggs, peanuts, tree nuts, fish, crustacean shellfish, soybeans, and wheat) are responsible for an estimated 90% of all food allergies on a world-wide basis.

The symptoms experienced by individuals with food allergies are variable on an individual basis and on the amount of the allergenic food that is ingested. Symptoms can range from hives and mild gastrointestinal upsets to life-threatening anaphylactic shock. More severe reactions are likely to occur upon exposure to a greater amount of the allergenic food. The product developer could be somewhat cavalier and claim that food labelling provisions will protect existing allergic consumers. However, labelling regulations in some countries could best be described as incomplete in their ability to protect allergic consumers. And, the introduction of a new allergenic food or component into an existing manufacturing facility opens the possibility for allergen cross-contact and tests the reliability of allergen control programs, allergen sanitation programs, and other costly provisions. The apparent increase in the prevalence of food allergies may be directly related to the more widespread use of common allergenic foods and food ingredients. Another issue involves ingredients derived from allergenic foods. The product developer must consider the allergenicity of such ingredients. For some ingredients such as casein and whey, the potential risk is obvious. For a few such ingredients (e.g. highly refined soybean and peanut oils), the risk is

acknowledged to be extraordinarily low. For a few such ingredients, including soybean lecithin, wheat starch, and fish gelatin, the level of risk is uncertain but probably low. However, a risk assessment should be made on each and every formulation where an allergenic food or an ingredient derived from a common allergenic food is considered.

Introduction of new allergenic food into a geographic area

The more widespread distribution of food products can also carry some allergenic risk into new areas of the world. For example, soybeans were introduced into the U.S. several decades ago and have now become a common allergenic food. Kiwi has been marketed much more widely in recent years with an accompanying increase in the prevalence of kiwi allergy. The more widespread use of certain known allergenic foods has led to an increased and more widespread prevalence of allergies to those foods. Several foods or food groups warrant some recognition because of the prevalence of reactions in certain geographic locales. Examples would include the sesame seeds, celery root, and buckwheat.

Introduction of novel food ingredients/proteins into the diet

Many novel food ingredients have high protein contents and some of these ingredients are derived from known allergenic sources. In other cases, the popularity and use levels of a novel food ingredient will result in an increase in consumer exposure to a potential allergenic source. In yet another scenario, a novel ingredient is included in the diets of infants or young children who may not have been frequently exposed to that potentially allergenic food or food ingredient previously. When a protein-containing ingredient is derived from a known allergenic source, some obligation exists to assess the potential allergenicity of the particular ingredient. This assessment probably involves clinical trials in groups of individuals with well documented allergies to the source food. Such experiments have been conducted for highly refined peanut oil and soybean oil. Similar trials are needed to assess the potential allergenicity of a host of other ingredients including fish gelatin, soybean lecithin, wheat starch, egg lysozyme, and lactose and lactoferrin from cows' milk. In these cases, the key consideration is often whether the ingredient contains sufficient residues of the known allergenic proteins to elicit reactions in sensitive consumers (e.g. oils, starch, lactose, lecithin). In other cases, the key consideration might be the prevalence of sensitisation to a minor allergen (e.g. egg lysozyme or bovine lactoferrin). A few novel food ingredients have high allergenic potential and should likely be avoided; cottonseed protein is the premier example. However, an increase in buckwheat consumption in Europe or North America would also likely elicit an increase in the prevalence of buckwheat allergy. Sometimes, a novel ingredient must be assessed for its potential allergenicity in much the same manner as might be considered for the novel proteins found in genetically modified foods (see below). Cottonseed protein isolate might be a good example. A product developer can create a situation where a particularly vulnerable segment of the population, namely infants and young children, are exposed to large amounts of a potentially allergenic protein. The historical example is the development of soybean infant formula.

Introduction of genetically modified foods

Genetically modified foods will usually contain novel proteins that should be assessed for their allergenic potential. If the selected gene is introduced from a known allergenic source, the assessment of the potential allergenicity of the novel protein is fairly straightforward and involves investigation of the binding of the novel protein to allergen-specific IgE antibodies obtained from allergic individuals. This sort of specific serum screening has been well accepted, although standard approaches for its application to genetically modified foods have not yet been developed. This approach was successfully used to demonstrate that a novel soybean variety modified for improved levels of methionine using a gene from Brazil

nuts contained a novel protein that was thus identified as the major allergen from Brazil nut; the company sponsoring this research abandoned the commercial development of this product. If the selected gene is introduced from a source with no history of allergenicity (the much more common circumstance), the assessment of the allergenicity of the novel proteins requires a different approach. Considerable scientific debate has occurred regarding the most appropriate way to conduct such assessments. Recently, the Codex Intergovernmental Task Force on Safety Assessment of Genetically Modified Foods recommended a weight-of-the-evidence approach that relied mainly upon an examination of the degree of sequence homology of the novel protein to a database of known food and environmental allergens and an assessment of the degree of pepsin resistance (most food allergens are resistant to pepsin hydrolysis). While most scientists agree with the application of these approaches, the decision criteria and the experimental approaches associated with these approaches have engendered some debate. For example, the Codex Intergovernmental Task Force suggests using a criterion of 8 contiguous, identical amino acids in the sequence homology comparison, while other groups have suggested using 6 contiguous, identical amino acids as the criterion. Additional approaches, including targeted serum screening and the use of animal models, have been recommended by some groups. The Codex Intergovernmental Task Force correctly concluded that these approaches were experimental at this time and could not immediately be applied in regulatory decision-making. However, these approaches may hold some promise and additional research is needed to examine their full potential. Finally, several other factors may be worthy of consideration. The level of expression (and thus consumer exposure) of the novel protein in the genetically modified food must be of some importance. Logically, susceptible individuals are much more likely to become sensitised to a protein that is present at high amounts in their diets than a protein that is present at minuscule levels. However, the threshold dose for allergic sensitisation is not known and no scientific consensus has been achieved in using exposure levels as one of the factors in the assessment of the allergenicity of genetically modified foods. Some comfort should be derived, however, from the fact that the novel proteins are expressed at very low levels in the currently approved varieties of genetically modified foods. Such will not be the case in the future as some genetically modified foods will be developed that will include substantial levels of novel proteins. In those cases, the assessment of the potential allergenicity of the novel protein will become a more important exercise. The development of additional approaches beyond sequence homology and pepsin resistance may be desirable before attempting to assess the allergenicity of this second generation of genetically modified foods.

Current practise in risk assessment: protecting all consumers!

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One may ask: 'is there a current practise in risk assessment of food allergy? Can we protect all consumers and what are the criteria for successful protection: preventing the most susceptible individuals from itches in the mouth or from severe reactions?' The risk assessment procedure includes hazard identification and characterisation, dose-response assessment, exposure assessment and risk characterisation. After having assessed the risk, managing the risk is the next step. There are two different scenarios to assess: the risk of sensitisation and the risk of eliciting an allergic response in a sensitised individual.

There are two different categories of potential food allergens that needs risk assessment

- Ordinary food allergens, e.g.
 - milk, egg, fish, peanut
 - hazelnuts, celery, peach, avocado (primary sensitisation may be pollen or latex)
 - (gluten)
- Novel food
 - genetically modified food
 - new ingredients and processes
 - newly introduced food

Ordinary food allergens

Sensitisation

It is known and generally accepted that some foods are allergenic and may sensitise susceptible individuals (hazard identification). Very little is known about the precise circumstances of sensitisation and risk assessment is seldom performed. There are three exceptions: children at high risk of developing allergy are advised to be breast-fed or have highly hydrolysed milk formula for the first 4 months of life to reduce the risk of sensitisation. In UK, mothers of high risk infants are advised not to eat peanuts during pregnancy and lactation, to breastfeed their babies for 4-6 months and not to feed their babies peanuts until they are at least three years of age (1). Gluten should not be introduced into the diet of young infants until 6 months of age in order to reduce the risk of developing celiac disease (not real allergy, but immune mediated). These risk management advices are a result of qualitative risk assessment based on the knowledge that avoiding an allergen will prevent sensitisation in the period of avoidance and that susceptibility to sensitisation may be greatest in the first months of life.

Elicitation

Every food store is full of food allergens but only a small fraction of the customers are at risk of having an allergic reaction from food. From the medical literature we know which food are allergenic (hazard identification). There has just very recently been consensus among clinical allergists that there is a threshold for allergic food reactions but there is no consensus about the actual threshold doses for different allergens (2). Therefore it is not possible to do a quantitative risk assessment i.e. to define the amount of allergen where there is no risk of an allergic reaction. The strict consequence of this is that if an ingredient is not on the label (and therefore can be avoided) the content in the food should be zero. Zero may be a very difficult figure to obtain and therefore some manufactures choose to label their products 'may contain

x' irrespective of an actual risk or not. From clinical cases of severe allergic reactions where the food has been analysed (3) and from challenge studies (2,4) we know that the lowest dose producing subjective symptoms is around 0.1 mg and that severe symptoms are caused by higher doses. (This may not be true in very susceptible young infants).

This knowledge is used in an indirect risk assessment/risk management strategy namely when detection kits for food allergens are developed. With the current methods very small amounts of allergens in a food matrix may be detected but in practise commercially available detection kits have a detection limit of 1-2 mg/kg. This approach very practically overcomes the zero and at the same time is able to identify allergens at a dose level where the risk begins.

As is apparent from the above the risk assessment procedure of food allergens are not very refined mostly because of lack of knowledge but also partly by the reluctance of the medical community to enter the discussion. Hopefully the discussion will continue to the benefit of the allergic consumer and to the guidance of the food industry.

Novel food

Whereas we accept that milk and egg are ingredients in the normal diet although they sensitise a part of the population and give rise to very serious clinical reactions we do not want new foods to be hazardous. This creates a serious challenge namely that risk of sensitisation from new food proteins should be assessed. The decision tree suggested at a FAO/WHO consultation in January 2001 concludes that if a protein introduced into a GM-food is a possible allergen (hazard identification) the development of the product should be discontinued (5). So here risk assessment has one step: hazard identification.

The possibility of predicting allergenicity of GM-food has been extensively discussed resulting in various decision tree approaches. It has more or less been taken for granted that assessing allergenicity of non-GM novel foods should be handled comparably.

The Micronesian nangai/ngali nut (*Canarium indicum*) has not been approved as novel food in the EU based on the conclusion that the allergenicity of ngali nuts has not been investigated and that adequate toxicological data are not available. In a later study 2/10 grass, birch and mugwort pollen allergic patients reacted to nangai nuts upon challenge (6). Several patients in this group probably have allergic reactions to other tree nuts. Is cross-reactions in pollen allergic patients an unacceptable risk or should a product like nangai nuts, that probably will have a limited consumption, be judged differently than GM-corn and GM-soy, which reaches the entire population including infants?

To answer the question from the start: yes, there are some practises of not very well developed risk assessment procedures. The debate on how to protect all consumers and the criteria for successful protection has just begun.

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Internal and external factors and criteria in decision-making

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The first hydrolysate-based product of Numico/Nutricia intended for the dietary management of cow's milk allergy was introduced more than 16 years ago. The internal and external criteria in deciding whether this product was adequate for the intended use and the quality control were assembled on a much more pragmatic basis than they are nowadays. However, progress to resolve the discrepancy between predicted performance on the basis of analytical data and clinical performance has been poor during the last 16 years. Moreover, regulating of e.g. hypoallergenic infant formulas via the EU infant formula guideline has not resulted in unambiguous guidelines and criteria for developing and testing these type of formulas. Because of the lack of detailed specifying target groups for clinical studies, studies have failed to demonstrate the efficacy of 'good' experimental products, while other studies falsely indicated the adequacy of 'questionable' products. Irrespective of the major efforts of experimental animal scientists to develop alternative models, the guinea pig model system of oral anaphylaxis remains the golden standard despite the numerous shortcomings of this model. New *ex vivo* studies/analysis using material from subjects with proven cow's milk allergy holds some promises for a better understanding, but these type of analysis are not suitable for routine testing both for practical and for ethical considerations.

In view of the unclear status both scientifically and also in the interpretation of the regulatory requirements, company internal factors are often at least as important in decision-making than the external factors. This makes the decision-making process less transparent because many internal factors are within the confidential domain. It is therefore desirable that the scientific world and the regulating authorities make an effort in defining more specifically the criteria for products intended for the prevention or therapy of food allergy. This process, however should be done in close collaboration with industry because too stringent regulations and high demands for large and expensive clinical studies will probably result in a decreased interest of industry to invest in the (further) development of specialised products for a relatively limited number consumers.

Regulatory approval: do we accept new cases of food allergy?

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The government of the Netherlands uses a differentiated approach in the protection of the allergy patient: allergy patients can get detailed information on the presence of possible allergens through a voluntary database system for food allergens. Information on the presence of allergens can also be obtained from the label of foodstuffs, which is required by legislation. This EU-based legislation has an urgent need for improvement. Protection of the allergy patient can be achieved through prevention of new cases of food allergy.

The Netherlands has established an Allergen Database (ALBA) in which data are compiled from industry and retailers on major allergens in branchnamed foodstuffs. The Netherlands Nutrition Centre, a government financed education centre publishes leaflets and operates an allergen telephone service for consumers. Also the medical field and dieticians are included in this project. Labelling rules are the competence of the European Union. The Netherlands share the view that due to a number of developments present labelling rules for allergens are not acceptable any more in providing sufficient information and protection of the allergy patient. Therefore a number of changes to directive 2000/13 has to be realised in due course. The European Commission has come forward with a proposal to this end.

The EC Novel Food regulation requires an authorisation procedure for novel food and novel food ingredients. This procedure includes especially genetic modified organisms and products thereof. Nearly all applications for GMOs have been filed in the Netherlands. The Health Council of the Netherlands is evaluating them. Evaluation of the allergenic potential is part of this evaluation. In the framework of the Codex Task Force on modern biotechnology a protocol has recently been elaborated on the estimation of the allergenic potential of GMOs.

Key problems are the presence of allergens through, so-called, carry-over, the absence of proper threshold levels and reliable and practical methods to identify the allergenic potential of a substance.

All efforts are directed towards making life easier and provide a better protection for the food allergy patient.

Development and testing of hypoallergenic formulas

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Hypoallergenic formulas are unique foods designed for use by patients who are allergic to the major protein components of their normal diets. Most of these patients are infants who have developed allergies to both cow milk and soy-based infant formulas. Hypoallergenic infant formulas (HIF) are made from sophisticated, expensive ingredients. They must be tested to insure very low residual food allergen content and to establish their clinical performance. 'Hypoallergenic' literally means 'less allergenic'. This description can be true when comparing cooked to raw foods, processed to unprocessed foods, or hydrolysed to unhydrolysed proteins. Management of food allergy in infants requires a more rigorous definition, which relates to the clinical performance of hypoallergenic formulas. Pediatric Societies and regulatory agencies now agree that 'hypoallergenic' formulas must not cause allergic symptoms in 90% of infants allergic to the base protein used to manufacture the formula, and that sufficient clinical research using double-blinded, placebo-controlled food challenges must be done to have a 95% statistical confidence in the allergy reaction frequency.

HIF are manufactured using protein hydrolysates selected for their minimal allergenic reactivity. Successful development of these products requires an understanding of the structure/function and dose/response relationships between food allergens and the immune system. Research in this area is complicated by the highly variable clinical manifestations of food allergy and the inability to comprehensively identify all food allergen epitopes. This makes it difficult to study food allergens directly. However, allergenic reactivity of a protein system is related to its immunogenicity (all allergens are immunogens), which in turn is related to protein antigenicity (all immunogens are antigens). It is therefore possible to accurately predict the allergenic potential of modified protein systems using much more standardised animal models of immunogenicity and immunochemical assessments of antigen content. These kinds of data are important prerequisites for the clinical challenge studies in allergic patients, which will establish the true hypoallergenic performance of HIF.

Development of HIF begins with research to identify a candidate hypoallergenic protein ingredient. This usually involves an immunochemical assessment of the degree of reduction in protein antigenic reactivity following hydrolysis. The antigen quantitation methods used for this purpose can also be applied to HIF quality assurance testing and may have general applications in the food industry. Currently the most widely used analytical method is the Enzyme-Linked ImmunoSorbent Assay (ELISA). ELISAs are used to measure intact antigens (to detect HIF contamination), and fragmented antigens (to characterise hydrolysates and HIF). Development of these assays requires an extensive knowledge of food allergen immunochemistry. There are a number of complicating issues: food allergens are complex mixtures of active proteins; specific allergen reactivity will be affected differently by various food processing methods including hydrolysis; the allergen epitope recognition pattern will vary among allergic patients and may also be different from the antigen epitope specificity of the antisera (usually animal-derived) used in the assay; allergens are not usually measured directly, antigen content is used as a surrogate; individual allergens are also complex with many recognition sites (epitopes) per allergen; data are reported as 'immunologically active (X)' based on standards and control samples. Food antigen ELISA methods are sensitive to

10 ng antigen / mL, with typical accuracies of \pm 5-20% and use either direct (sandwich) or inhibition formats. ELISA results can easily differentiate clinically hypoallergenic products from more allergenically active products based on partially-hydrolysed proteins.

Animal models also play an important role in the development of HIF. An early model developed to assess food allergen reactivity was the oral sensitisation guinea pig model. Generally, this model has demonstrated variable sensitivity and in some cases fails to identify clinically reactive products. A preferred model uses the antibody response of hyperimmunised rabbits. This model has successfully differentiated non-hypoallergenic products based on partial hydrolysates which were highly reactive, versus HIF which were very weakly reactive, versus non-reactive products made from only free amino acids. None of the available models accurately describe the IgE mechanisms of human food allergy.

Commercial production of HIF presents its own set of challenges. HIF must be free of allergen contamination. HIF allergen quality assurance programs begin with ingredient qualification and include manufacturing equipment cleaning validation, manufacturing process control, and finished produce release testing based on antigen content. The ELISA methods used during HIF product development are also useful here. The analytical testing goal is to insure HIF products are free of 'immunologically significant' levels of antigens. How much antigen is 'immunologically significant'? There is not an agreed standard. This depends on the 'strength' of the allergen, its denaturation and degradation state, allergen solubility and digestive processing, and many host factors. Recent data indicate a substantial difference in reaction thresholds between cow milk and soy allergens. Data from HIF clinical studies are not comprehensive but indicate that 50-150 μ g of allergen/feeding might be in the 'hypoallergenic range', with 99% non-reactive at levels of 5-15 μ g of allergen/feeding. Manufacturing HIF that successfully achieve these low allergen levels provides a useful model for food allergen control illustrating methods to avoid contamination, confirm production equipment cleaning, and judge product quality. An example of equipment cleaning validation using immunochemical data is presented.

Possibilities and limitations of agricultural GM crop-products

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Foods derived from genetically modified (GM) crops that were enhanced through the intended introduction of functionally specific DNA have been in the U.S. market since 1994. Most of the current GM crop traits reduce the need for applying chemical pesticides as they specifically protect the crop against insect pests, or allow reduced tillage practices because they confer herbicide tolerance to the crop. These input traits also offer greater production yields, lower production costs and improved commodity quality. Some of the GM crops, such as the Flavr Savr™ tomato with delayed softening allowing the fruit to ripen longer on the vine, providing enhanced food quality for the consumer. However, many of the benefits of GM crops are hard to quantify and are not widely known or appreciated by the lay public. These include global environmental impacts such as the reduced use of chemical pesticides, reduced use of fossil fuels, reduced soil erosion, and enhanced food qualities such as the reduction in the concentration of specific mycotoxins and of possible allergens such as the pathogenesis related proteins produced by plants in response to injury. New products under development include nutritionally enhanced foods and plants that synthesise pharmaceutical agents or industrial raw materials. An overview of the current and near-term future products will be discussed.

Current products

Globally, the total agricultural land used to produce the GM commodity crops has increased from approximately 1.7 million hectares in 1996 to 52.6 million hectares in 2001 (1). Based on current (2001) farmland production estimates, 63% of the soybeans, 19% of maize, 13% of cotton and 5% of canola (oilseed rape) are grown from GM seed. While the GM market share of a number of other crops is small, there are approvals or field permits for a number of different fruits, vegetables, fibre crops and ornamental plants. Most of the current GM crop production is in the USA (68%), Argentina (22%), Canada (6%) and China (3%). Approximately three-quarters of GM crops are herbicide tolerant, nearly one quarter are resistant to specific insects and most of the others are resistant to specific plant viruses.

Most approved GM product trait characteristics are due to the production of low levels of protein. For example, Cry1Ab in insect protected maize is present at only 1-8 ppm while CP4 EPSPS in glyphosate tolerant soybeans is present at ~200 ppm. Some GM product traits are due to the presence of a specific RNA, such as the PLRV replicase RNA, which confers resistance to infection by this virus. The safety assessment of GM crops is quite extensive, usually involving comparative studies of the modified crop with current commercial varieties with regard to composition, nutrition, environmental impact, and potential allergenicity.

Future Products

Rapid advances in genomics, biochemistry, plant physiology and medicine are opening the door to an expanding array of new products, some of which will be discussed. A number of these products are already in limited, regulated field trials; some are only in the greenhouse, while others are in early stages of development.

Many of the most exciting new products will come from the use of plants as factories to produce high purity, high value products more efficiently and cheaper than can be accomplished through current conventional manufacturing processes. Most of these products will be produced on a very limited scale, with strict control on the distribution of seed, production location, product and residue distribution.

Pharmaceutical products

Therapeutic humanised monoclonal antibodies and protein growth factors are under development. These antibodies cannot be produced in sufficient quantities (hundreds of kilograms) in mammalian cell culture systems and are not processed correctly by microbes to make bioactive molecules. However, various plants, including corn and tobacco have been modified to produce antibodies, and some have been tested in clinical trials. Their potential products include hormones, or modified hormones such as taxol.

Raw materials

Industrial enzymes that may be produced in plants could include enzymes similar to subtilisin or other proteinases, lipases or cellulases. Some of these would have to be produced by the plant in the inactive form and activated following purification. Oils and waxes that are currently produced from animal products, from endangered or rare plants or from fossil fuels may be produced efficiently in modified corn, soybeans canola or sunflowers. Cotton, flax and wood fibres may be modified by the addition of enzymes that improve the characteristics of naturally occurring plant fibres. Alternatively, whole new pathways may be introduced into a plant to make biodegradable plastics or possibly spider silk, either of which would be useful as raw materials for a wide spectrum of products.

Human nutritional products

Nutritional supplements are obvious targets for consumer products and may offer an inexpensive way to improve nutrition. For example, vitamin A deficiency affects an estimated 200 million people in Asia. Vitamin A is essential for preventing night-blindness and improves immune function. Two GM crops developed to provide the needed precursor for vitamin A, beta-carotenes, are golden rice and golden mustard. Another example of a nutritionally enhanced GM crop is soybean producing omega-3 fatty acids. The consumption of dietary omega-fatty acids is thought to reduce the risk of cardiovascular disease. Food crops such as maize, corn and sweet potatoes could be enhanced to include higher protein content to meet nutritional needs without increasing the consumption of animal products. Higher oleic acid cottonseed, soybeans and canola (oilseed rape) would provide a better ratio of monounsaturated fatty acids, which are thought to lower the risk of cardiovascular disease.

Food allergen reduction

While speculative, the potential is here now to reduce the allergenicity of specific food proteins by different modifications. Allergen levels can be reduced by post-transcriptional gene-silencing. Reducing the number of disulphide bridges may destabilise highly cross-linked stable allergens. Specific IgE epitopes may be modified to reduce the affinity of binding and the likelihood of reactions.

Animal feed

A significant fraction of the soybean and maize crop in the USA and South America is fed to animals for the production of meat, milk and eggs. The macronutrient content of these crops could be altered to enhance animal production, with more nutritionally complete diets and without using the supplements that are routinely added. Potential products include maize with higher protein content, maize with increased amounts of bioavailable essential amino acids, and forage crops with greater *in vivo* digestibility.

Agricultural productivity

World population is expected to increase by 2.5 billion people in the next 25 years. Concomitantly, the food requirements for this growing population must increase. Unfortunately there has been a decline in the annual rate of increase in cereal yields such that the annual rate of yield increase is below the rate of population increase (2). In order to feed this growing population, crop yield will have to be increased and some of the increase in yield will be due to genetic engineering of foods. Many projections include the need to increase production of food and feed plants by 45 to 75% over the next 18 years in order to meet demands. Traits currently under development include increased tolerance to drought and to high salts, better disease resistance including antiviral, antifungal and antibacterial products. More diverse insect protection traits are also expected to reduce the need for chemical pesticides, and the continued use of herbicide tolerant crop varieties will allow maintenance of the soil structure on current farmland.

Safety

Future GM products will have to go through the same rigorous safety assessment that is currently in place. Certain future products will require additional steps on a case-by-case basis, to evaluate specific toxicity, allergenicity or other concerns arising from the characteristics of the GM trait. One of the areas that all of the major biotechnology companies are working on, and the regulatory agencies around the world are evaluating, is how to improve the assessment of the potential allergenicity of any new protein. As our understanding of the immunology, biochemistry and in particular of the science of diagnosing specific allergies are improved, the assessment can be improved. The potential utility and predictive value of various animal models of allergy is another tool that is being considered for the allergenicity assessment process.

The potential future products discussed here could be commercial within 10 to 15 years, and provide a wide range of benefits. Beyond that, the potential benefits that could be derived from agricultural biotechnology are limited primarily by our imagination.

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The role of the intestinal flora in food allergy

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Microbial exposure, including infections can have long-lasting non-specific systemic effects on the nature of the immune response to unrelated antigens. Recently particular interest has focused on the potential role of the intestinal microflora. The perspective of the human colon in health and disease is by no means a new concept as Metchnikoff already more than a century ago indicated the clinical importance of the host colonic microflora. Rapid advancements have been made, particularly over the past 10 years and the bacterial microflora of the human gut is now accepted as an integral component of the host defence system and has generated considerable interest in the functional food/nutraceutical industry.

Gut flora

The total mucosal surface area of the adult human gastrointestinal tract is up to 300m², making it the largest body area interacting with the environment. It is colonised with over 10¹⁴ micro-organisms, weighing over 1 kg and corresponding to more than 10 times the total number of cells in the body. The gut-associated lymphoid tissue is continuously confronted with a vast array of antigens, ranging from food antigens to pathogenic micro-organisms. The gut immune system has the capacity to distinguish between potentially harmful antigens, e.g. microbes and harmless antigens, e.g. foods. The former induces strong mucosal and systemic immunity and the latter immunologic (oral) tolerance. The difference between the two types of antigens is probably related to how they are presented by antigen presenting cells. Thus, microbial antigens are mainly presented by macrophages capable of phagocytosing whole bacteria. Phagocytosis stimulates the cells to express of surface bound T cell co-stimulatory molecules and the secretion of cytokines capable of activating T cells.

The gastrointestinal tract of the new-born baby is sterile. Soon after birth, however, it is colonised by numerous species of micro-organisms. Colonisation is complete after approximately one week, but the numbers and species of bacteria fluctuate during the first three months of life. When the microflora has been established it is surprisingly stable over time and environmental changes, e.g. a treatment period with antibiotics, usually changes the composition of the microflora only temporarily. The gut flora is thus the quantitatively most important source of microbial stimulation and may provide a primary signal for driving the postnatal maturation of the immune system, thus inducing Th1-like immunity. Animals lacking a normal intestinal microflora display enhanced immunity to fed proteins, as compared with conventional animals. Rook and Stanford suggested two major syndromes that could be the result of inadequate microbial stimulation early in life, i.e. inadequate priming of T helper cells, leading to an incorrect cytokine balance and a failure to fine-tune the T cell repertoire in relation to epitopes that are cross-reactive between self and micro-organisms. The authors coined the expressions 'input deprivation syndrome' and 'uneducated T-cell regulation syndrome'.

Microbial ecology and allergy

There are considerable differences in the composition of the gut flora of healthy infants in Estonia, with a low prevalence of allergy, and Sweden with a three-fold higher prevalence of

positive skin prick tests among children. For example, *Lactobacilli* and *Eubacteria* were more common in Estonian than in Swedish infants, whereas the reverse was true for *Clostridium difficile*. Furthermore, the postnatal colonisation seems to be more intense in Estonian than in Swedish babies as the counts of aerobes, particularly staphylococci, enterococci and enterobacteria are much higher during the first week of life in the former. These differences are interesting, as Estonia is a country with a Northern European culture where, due to the 50-year occupation by the Soviet Union, the life style in many ways is similar to what prevailed in Scandinavia 30-40 years ago.

Differences in the composition of the intestinal microflora have also been demonstrated between allergic and non-allergic two year-old children, both in Estonia and Sweden. Thus, bifidobacteria were more prevalent in the latter, while the counts of coliform bacteria were higher in the atopic children. Two recently published prospective studies have confirmed that bifidobacteria are less commonly found in infants who develop allergic disease than in those who do not. In one of the studies, the counts of bifidobacteria tended to be lower at three weeks but not later in infants who developed allergic manifestations during the first two years of life. These infants also had significantly higher counts of clostridia at three weeks than the non-allergic babies. In the second study, 21/26 healthy infants harboured bifidobacteria during the first month of life and 25/26 during the first three months, as compared to 8/18 and 9/18 infants who had developed allergic disease by age two.

An alternative approach to study the microbial flora is to investigate the functional status of the flora, i.e. 'what have the microbes done'? A microflora associated characteristic (MAC) has been defined as any anatomical, physiological, immunological or biochemical function in a macro-organism, which has been acted on by the microflora. Examples of MACs include the formation of short chain fatty acids (SCFAs) by anaerobic microbes in the human colon, microbial transformation of cholesterol to coprostanol, and the microbial inactivation of intestinal trypsin which can be measured by the remained faecal tryptic activity (FTA) in faeces. This approach to study the microbial ecosystem in the gut has several advantages over more traditional analyses based on isolation and enumeration of the micro-organisms, as the latter are expensive, time consuming and associated with problems in obtaining samples from parts of the GI-tract that are normally inaccessible.

Employing this approach, an analysis of short chain fatty acids was performed in stool samples from 25 allergic and 47 non-allergic Swedish infants. Iso-caproic acid was detected almost exclusively in allergic infants. This compound is associated with the presence of *Clostridium difficile*. In contrast, the levels of several fatty acids associated with a *Lactobacillus* flora were higher in the non-atopic infants.

Probiotics and immune function

Certain microbes have been suggested to promote human health and even to prevent disease. Such probiotic bacteria have raised considerable interest in the dairy industry and the public. Unfortunately much of the research in this field has been sponsored by industry and focused on a particular strain. There is however some support for a beneficial effect of certain bacteria on human health, although little is known whether any of these effects are strain specific or common for one or several species.

A recent prospective placebo-controlled study lends support for an influence of the microbial gut flora on allergy. In this study, a strain of lactobacilli or placebo was given to pregnant mothers before delivery and then for up to 6 months while breast feeding and to babies who were not breast fed. The treatment was associated with a lower incidence of atopic dermatitis during the first two years of life in the infants. The findings need confirmation however in a

larger and differently designed study.

It has been suggested that certain strains of lactobacilli can inhibit allergen induced IgE production by murine splenocytes, possibly through induction of IL-12 secretion by macrophages. Thus, the intestinal flora may play a crucial role in generating Th2 cell populations fully susceptible to oral tolerance induction. Furthermore, the severity of atopic eczema improved in infants treated with extensively hydrolysed whey formula fortified with lactobacilli. The study groups were small however and the study period was only one month. Interestingly, products from *Lactobacilli* degraded casein proteins from cow's milk have been shown to stimulate or inhibit lymphocyte proliferation and IL-4 production, and to enhance IFN γ production. In mice fed lactobacilli, the spleen cells produced predominantly Th1 cytokines like IFN γ and IL-2, while the production of Th2-associated cytokines such as IL-4, IL-5, IL-6 and IL-10 were lower than in the control mice.

Conclusions

Several studies indicate an imbalance in the gut flora of allergic infants. As the microbial flora is driving the maturation of the immune system and is a prerequisite for development of oral tolerance, changes in its composition, as a consequence of an altered lifestyle and diet in industrialised societies, may conceivably play a role for the increasing prevalence of allergy. All these findings could suggest that differences in the indigenous intestinal flora might affect the priming of the immune system in early childhood and that the observed differences between allergic and non-allergic children are not secondary phenomena.

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Can specialty products prevent food allergy?

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Food allergy currently affects approximately 2% of the population and appears to be increasing. At the present time there are no effective therapies for IgE-mediated food allergies, and patients must be instructed in food-allergen avoidance and self treatment after accidental ingestion. Probiotic therapy has attracted the renewed interest of clinicians and basic investigators from a variety of traditional research disciplines. Probiotics are live microbial food supplements or components of bacteria, which have beneficial effects on human health. Probiotic ingestion can be recommended as a preventative approach to maintaining the balance of the intestinal microflora and thereby enhance "well-being". Undoubtedly, probiotics will vary in their efficacy and it may not be the case that the same results occur with all species. Those that prove most efficient will likely be strains that are robust enough to survive the harsh physicochemical conditions present in the gastrointestinal tract. This includes gastric acid, bile secretions and competition with the resident microflora. Oral introduction of probiotics can help in treatment of food allergies by alleviating intestinal inflammation. It has also been suggested that intestinal micro-organisms could down regulate the allergic inflammation by counter-balancing T-helper cell type 2 responses and by enhancing antigen exclusion through induction of an IgA response. In addition, probiotic bacteria may promote endogenous barrier mechanisms in the patients with atopic dermatitis and food allergy. These results suggest that probiotics may act as a useful tool in the treatment of food allergy. However, the mechanisms underlying the observed modulations of the immune system are largely unknown.

Since it is very difficult to study sensitisation in humans we have developed an oral Brown Norway rat food allergy model. After oral exposure of the animals by daily gavage dosing, without the use of adjuvants, the animals develop specific IgE antibodies. In addition, immune mediated effects, like an increased gut permeability, drop in blood pressure and breathing frequency and increased release of Rat Mast cell Protease II, can be observed in these animals upon oral challenge. Using this animal model the possible therapeutic and/or prophylactic properties of probiotics will be investigated.

Threshold levels for challenge reactions: how much is too much?

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Risks of developing allergic reactions depend on the individual's sensitivity and the level of exposure to the allergen. For risk assessment, information about the exposure of food allergic patients to certain doses of the offending food needs to be combined with information about the distribution of threshold levels for eliciting allergic reactions in the allergic population. Individual threshold levels are best determined by double-blind, placebo-controlled, food challenges (DBPCFC) with gradually increasing allergen doses.

Recently we performed two threshold studies with peanut and hazelnut, respectively. Peanut is one of the most common food allergens. The prevalence of peanut allergy in adults is estimated at 0.48% in the UK and together with tree nut allergy 1.1% in the USA. Peanut is a member of the legume family, but, although extensive *in vitro* crossreactivity occurs among legumes, clinically significant crossreactivity is rare. Not all allergic patients are as sensitive and will react to very low doses of peanut protein. However the risk of developing an allergic reaction after ingestion of a certain peanut dose is not known. Hourihane et al. performed double-blind placebo controlled food challenges to reveal very low threshold doses in 14 adults. Objective symptoms occurred after a dose as low as 2 mg and subjective symptoms already after a dose of 100 µg of peanut protein.

In our study we also performed DBPCFC with increasing doses of peanut meal in 26 adult patients with a convincing history of peanut related symptoms. All patients reacted to the challenge. Threshold doses for allergic reactions ranged from 100 µg up to 1 g of peanut protein. Patients with a history of severe symptoms had lower threshold doses compared to patients with mild symptoms ($p < 0.0001$). Our results show that 50% of a peanut allergic population will already suffer from an allergic reaction after ingestion of 3 mg of peanut protein (about 1/50 peanut !!).

There is little information concerning threshold doses in hazelnut allergic patients. Yet, hazelnut allergy is common in Western Europe, mainly as part of the para-birch syndrome. Symptoms vary from the oral allergy syndrome to gastro-intestinal symptoms and wheezing but even fatal reactions have been described, mainly in children and adolescents. Doses of 7.2 and 10 mg of hazelnut protein have been described to induce respiratory or gastro-intestinal symptoms in allergic patients. The ubiquitous use of both roasted and unroasted hazelnuts in pre-packaged foods further emphasises the need to evaluate the sensitivity of allergic patients to hazelnut.

In thirty-one patients with a convincing history of hazelnut-related allergic symptoms a DBPCFC was performed. Twenty-nine patients had a positive challenge. Threshold doses for eliciting subjective reactions varied from a dose of 1 mg up to 100 mg hazelnut protein. Objective symptoms were observed in two patients after 1 and 1000 mg, respectively. Extrapolation of the dose-response curve showed that fifty percent of our hazelnut allergic population will suffer from an allergic reaction after ingestion of 6 mg of hazelnut protein.

These data show that the threshold levels for peanut and hazelnut are low. This stresses the need for careful labelling of food products and strategies to prevent and detect contamination of food products with peanut and hazelnut residues. The importance is stressed even more as patients suffering from severe reactions will react to lower doses than patients with mild symptoms.

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Detection of food allergens: how low can we go?

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Immunochemical methods for detection and quantification of food proteins have been developed since the 1970's. With food allergy becoming more important for consumers and food companies, similar tests were developed for certain allergens. Many different experimental set-ups have been described, and some of them appeared to be applicable for the food industry. Based on those techniques, test kits for peanut, egg, wheat, and milk are now commercially available.

Aspects that are important for allergen tests are specificity and sensitivity. Since some allergens are genetically closely related, specificity is sometimes a problem. An example is the cross-reactivity on immunochemical level of certain nuts. The sensitivity issue is related to thresholds for allergens in patients. Recently, thresholds for peanuts have been described for a selected number of patients, and additional data are underway. For allergens other than peanut, only limited information is available, mainly obtained from case reports. Provided that clear threshold values are present, the technological challenge is to adjust the sensitivity of the test to the desired level.

This presentation deals with the above issues, and aims to inform the food industry on the possibilities and limitations of allergen testing.

Exposure assessment: do we have any idea on intake?

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University of Southampton, UK

Using double blind food challenges it has been possible to estimate a *reactivity* threshold dose for some of the common allergenic foods: peanut, egg and milk. One report stated that 0.2 mg (of egg) caused an allergic reaction in a double-blind food challenge. Other foods are being studied using similar methodology. In all series reported so far at least a single subject has reacted to the first dose so no *safety* threshold can be identified confidently at present. Future studies need standardised or comparable methodologies to allow valid comparisons to be made between foods regarding threshold doses and amounts consumed.

There are many circumstances surrounding allergen exposure in the community, which may affect a subject's reactivity. These may include external factors such as the time of year (whether in or out of pollen season), location (most significant exposures take place outside a home setting) and availability of rescue medication. Intrinsic factors (related to the patient) include decision making processes (often poor decision making contributes to allergen exposure), consumption of possible adjuvants for an allergic reaction (alcohol) with the offending food and the co-existence of illnesses (asthma) or use of medications (non-steroidal anti-inflammatory agents) which may exacerbate an allergic reaction.

The most important factors affecting severity however are likely to be the nature of the allergen and the dose consumed. Peanut and tree nut dominate the reports of fatal and near fatal allergic reactions to foods. Other foods are also cited as having caused fatalities but at a very low rate compared to peanut and tree nut.

It is difficult to accurately quantify doses that have elicited allergic reactions in community settings. Any analysis of these exposures may take place after the exposure event or maybe even post mortem. However, community reactions have been reported to 0.52% (wt/wt) for weight of milk protein or 200 mg of whey protein.

Self-reports from peanut individuals suggest that a significant proportion have, usually mild, reactions to inhaling peanut protein. In one series 17% of peanut allergic individuals report such sensitivity and in another series 14/48 (29%) allergic reactions after exposure to peanut or tree nut on aeroplanes, were in response to inhaled peanut with no other obvious exposure that could account for the symptoms. It is difficult to imagine how low might be the actual dose of protein involved in these reported reactions.

The use of standardised methodology for formal challenges may allow thresholds to be estimated for more foods in future. Assessment of actual amounts implicated in community exposures may have considerable forensic and legal implications. Confident interpretation of such evaluations will depend on the development of robust and reliable methods to detect allergens in complex food matrices because most severe reactions in the community are to hidden rather than obvious allergens.

Changes in practice of risk assessment: from possibility to probability

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In the safety or risk assessment for chemical substances in food, two different approaches are usually applied: one for effects for which a threshold in toxicity is assumed to exist (applicable for most types of toxic effects) and one for effects for which a threshold in toxicity is not assumed to exist (like for genotoxic carcinogenicity). For food proteins being capable of inducing food allergic reactions, neither of the approaches is generally applicable without running into practical problems. Development of specific methodologies for risk assessment for allergenic food proteins is a focal research area of TNO Nutrition and Food Research. The ideas and background, the ultimate goals of this research program, and some approaches in risk assessment for allergenic food proteins are described in this paper.

Safety and risk assessment for chemicals in food

In the safety or risk assessment for chemical substances in food, two different approaches are usually applied. The first approach is applied for effects for which a threshold in toxicity is assumed to exist. This is applicable for most types of toxic effects. For these effects, the highest intake level of a chemical at which no adverse effect is known to occur in animals or humans (No-Observed-Adverse-Effect-Level or NOAEL) is established. From this NOAEL, a health based intake limit (e.g. an Acceptable-Daily-Intake or ADI) is derived by applying an appropriate safety factor or margin. An actual intake at or below this intake limit is considered to be safe. Such an intake limit can also be used to establish concentration limits for food products. For effects for which a threshold in toxicity is not assumed to exist, like for genotoxic carcinogenicity, this NOAEL/safety factor approach is not applicable. For genotoxic carcinogens, any exposure to the substance is assumed to result in an additional risk for cancer. For substances causing such effects, exposure should preferably be as low as reasonably achievable. For practical reasons, a certain low additional cancer risk is often accepted (e.g. 1 additional case of cancer over a lifetime in a population of 10^6 persons), giving guidance for efforts in limiting the amounts of such substances in food and thus the exposure. The exposure level causing only such a low additional cancer risk is usually estimated by linear extrapolation on the basis of observed tumour incidences in animals or humans exposed at much higher doses. For food proteins being capable of inducing food allergic reactions, neither of these approaches is generally applicable without running into practical problems.

Safety and risk assessment for allergenic food proteins

Safety assessment for allergenic food proteins

Only limited information is available on thresholds with respect to allergenicity of food proteins. Yet, there are no scientific arguments to assume that such thresholds will not exist. Particularly for already sensitised individuals, scientific studies indicate thresholds for effect elicitation. But also for sensitisation, scientific information suggests the existence of dose-response relationships and thresholds. The existence of thresholds enables possibilities for a threshold approach in safety assessment. However, additional research and method development will be needed to establish the actual thresholds. Moreover, decisions will have

to be made regarding the desired statistical confidence and level of protection.

Although such a threshold approach may be applicable for the assessment of certain situations, for instance in setting limits for contamination with allergens or for labelling requirements, a threshold approach would most likely pose some major practical problems in other situations. It is likely that a threshold approach will result in very low limits, which would restrict the opportunities for new product and process developments. In this respect, it should be considered that most, if not all, foreign proteins have an allergenic potency. As such, every protein in our daily diet may induce allergic reactions in some individuals. Yet, we accept the presence of allergens, including major allergens, in our daily diet and we even feed part of our offspring with one of the eight major sources of food allergens from the first day after birth onwards: cows milk. On the other hand, we know that about 90% of all documented food allergies world wide are due to only 8 main (groups of) allergens (i.e. peanut, soy, tree nuts, wheat, milk, egg, fish, and crustacea). This suggests that, apparently, many proteins in our daily diet pose no or only a minor concern. Allergenicity of (new) proteins or (new) protein containing products in our food supply therefore needs to be considered in a proper perspective.

Worst case risk assessment for allergenic food proteins

Risk assessment is one tool that can be used in putting allergenicity of (new) proteins or (new) protein containing products in our food supply in a proper perspective. In this respect, it is important to realise that also in risk assessment, differences in focus may occur. For instance, the question whether or not an adverse event may occur is another than, and requires another approach in comparison to a question what the chance of an adverse event (of a certain type or seriousness) may be. To illustrate this, a hypothetical case is described. Let's assume a case of contamination of candy bars with an allergen, at a contamination level of maximally 0.03 mg per candy bar. Let's further assume that in one package, 3 candy bars are contained. Can we exclude an allergic reaction? To answer this question, we need to know the trigger thresholds for patients. Although information in this respect is limited, literature data indicate that food allergic reactions may occur with allergen doses of 50 to 100 mg or higher. However, recent research indicates lower thresholds. For instance, research with allergic patients by the University Medical Centre of the Utrecht University, Utrecht, the Netherlands, in collaboration with TNO Nutrition and Food Research, demonstrated that allergic reactions may occur already with hazelnut protein doses of 1 mg and peanut protein doses of 0.1 mg. The dose of 1 mg hazelnut protein was the lowest dose tested, whereas with a dose of 0.03 mg peanut protein no effects were reported. With answering the question whether we can exclude an allergic reaction, we need to take into account that one allergic individual may use several candy bars within a short period of time. In view of the packaging concept, the use of 3 candy bars seems reasonable as a (worst case?) starting point for risk assessment. On the basis of this and the maximal contamination level, an intake of around 0.09 mg cannot be excluded. In view of this possible intake scenario and the recent research results, i.e. a threshold between 0.03 and 0.1 mg or below, we cannot exclude that an adverse event may occur. However, what is the chance that this may occur? To answer the latter question, another approach will be needed, taking into account, among others, that the sensitivity of the individuals that may consume the contaminated candy bars may differ from the highest sensitive individuals in the clinical studies, that not all individuals may consume all the 3 (some less, but on the other hand some possibly more) candy bars, and that not all candy bars will be contaminated up to the maximal level.

Probabilistic risk assessment for allergenic food proteins

As a tool in this process, TNO Nutrition and Food Research aims at a probabilistic approach to finally describe the actual risk that we may encounter with various situations and scenarios. Besides for the assessment of situations where a limit set on the basis of a threshold approach is exceeded or where no such limits apply, probabilistic modelling may

be used in the validation of established health based intake limits and to determine the statistical confidence and level of protection with such limits.

The ultimate goal of probabilistic modelling in risk assessment for allergenic food proteins is to determine and describe the probability that an adverse event (of a certain type) should be expected to occur. This probability is determined by many chance determining factors, such as the chance of a person becoming (or having become) sensitised, the likelihood, level, and pattern of intake of products containing the allergen, and various other circumstantial factors. In probabilistic modelling, it is tried to estimate the overall probability and statistical confidence of a situation to occur by using information on probabilities or probability distributions for the various chances determining factors.

Model development

An example of a simple model is given in the enclosed figure. In this example, it is illustrated how information on thresholds for effect elicitation in sensitised individuals, information from food consumption surveys, and results from analyses on allergen levels in foods may be used to estimate the chance on an allergic reaction in a population of sensitised individuals. In this model example, a representative use pattern by allergic subjects of products with a certain level of contamination with the offending food allergen is assumed.

Such a model can be refined and extended by using as much information as available. For instance, not all individuals that may consume the candy bars from the case above will have an allergy, while only part of individuals with an allergy or allergies will suffer from food allergy. Again a smaller proportion of individuals will be sensitive for the specific food allergen that has contaminated the candy bars. Examples of aspects that can be taken into account in the refinement and extension of models for probabilistic risk assessment for allergenic food proteins are given in the enclosed table.

Figure: Example model development for probabilistic risk assessment

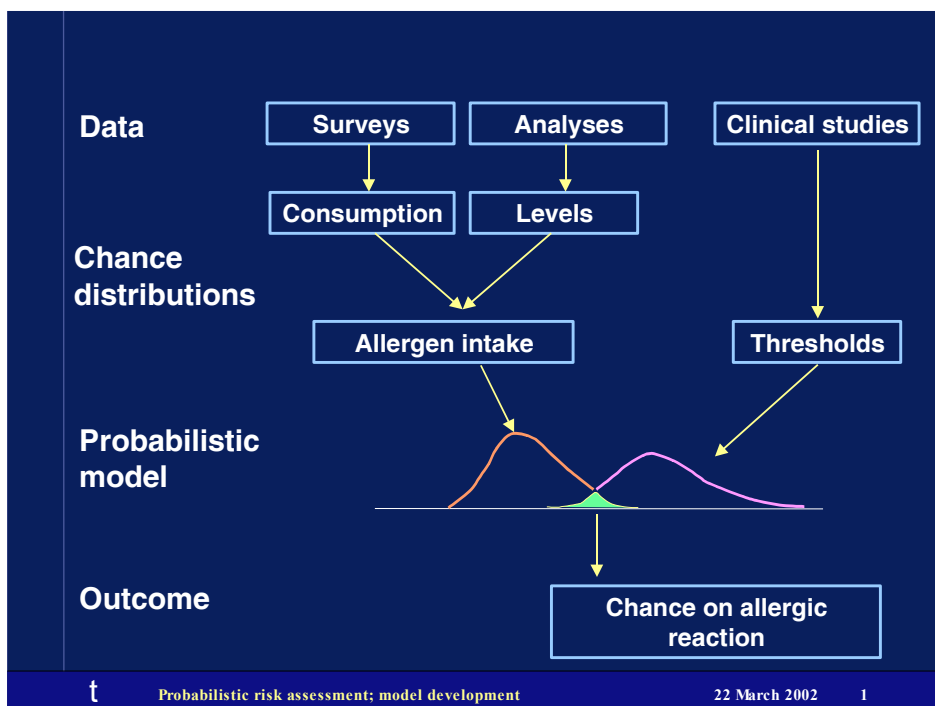


Table: Aspects that can be taken into account in probabilistic model refinement and extension.

-
- prevalence of atopy (point estimate)
 - prevalence of food allergy (point estimate), either or not with distinction made between children and adults
 - distribution of prevalences of allergen-specific food allergies, either or not with distinction made based on e.g. levels in foods
 - physico-chemical and/or biological characteristics, like
 - digestibility
 - stability in processing and preparation
 - effects in screening (e.g. animal) models
 - distributions of thresholds for different allergens (from a worst case allergen assumption towards probability), either or not with distinction made based on screening results
 - distributions of thresholds for different effects, e.g. based on severity or consequences
-

Application of a probabilistic risk assessment approach: from possibility to probability

Probabilistic modelling in risk assessment for allergenic food proteins should be regarded as a step-by-step reduction in and/or description of uncertainties in the risk assessment. It is applicable already when information on the probability distribution for one or several chance determining factors is available and it can be refined and extended continuously with new information becoming available. In collaboration between various departments of TNO Nutrition and Food Research as well as with the University Medical Centre of the Utrecht University, studies are being undertaken to develop tools and generate data that can be used in probabilistic modelling in food allergen risk assessment. Although still with uncertainties, the ultimate goal of this research is a better description of the risks of the presence of allergenic proteins in our food and thus a better starting point for risk comparisons, decision making, and risk management. Not so much the possibility as such, but rather the probability of occurrence of an adverse event and its severity and consequences are thus to be considered and put into a proper perspective in the ultimate decision making and risk management.

HACCP to control allergens in food manufacturing processes

D. Cromie

Unilever Bestfoods, UK

Unilever's approach to controlling allergens is to ensure that consumers who know they are sensitive to common allergens, can avoid inadvertent exposure to those allergens. This is achieved by informing consumers about the presence of allergens in Unilever products.

In order to achieve a consistent measure of allergen control at a corporate level, Unilever has defined both the scope and standards required for dealing with allergens, which ultimately provide the terms of reference for control in manufacturing, via HACCP. In order to do this Unilever formed a central team of experts to formulate and deploy the global standards. Within these standards, Unilever differentiates between common and rare allergens, 'common' being those substances which are common causes of allergic reactions in the markets where the product is sold, and 'rare' being those allergens not prevalent in the markets where the product is sold. The 'common' allergens align with the Codex standard, and also take account of documented regional allergens, e.g. celery in the EU. The Unilever global standard focuses on the common allergens. All Unilever organisations globally are in the process of implementation.

Control of allergens in the absolute sense is unlikely, because of complexities in the supply chain, a lack of knowledge of threshold values, the design and age of installations, and because of the long list of potential allergens. The prime objective is to identify the risks and reduce this based on ALARA principles (As Low As Reasonably Achievable).

The HACCP principles can be used to identify, eliminate and moderate risk. Elimination, segregation (physical or time-bound), validated cleaning regimes, reliable in-factory procedures, change control and accurate labelling are all key. The project must be supported at a senior level in the business, given the likely on-costs.

Allergens: why do we need to label?

N. Craddock

Nestlé, UK

The serious reactions, and even fatalities, which can arise in particular from peanut allergenicity have caused the food industry to consider at great length what steps can, and should, be taken to reduce further the risks to which a small minority of the general public are exposed. Although similar reactions can be caused by other allergens, the major effort has been devoted to peanuts and tree nuts but, as our awareness of food allergenicity has increased, so also have the implications for industry. This presentation will examine a number of fundamental legal, practical and commercial issues that need to be considered, and the statutes from which liability may derive.

All food manufacturers have an over-riding legal responsibility to ensure that their products are safe and fit for the purpose intended. They must also comply with relevant food labelling legislation. However, in addition to aspects of food law, it is also necessary to consider the implications of product liability, product safety and other civil law requirements.

In practice, the requirements of food law are relatively straightforward but the extent to which a manufacturer may be held liable under other statutes or under civil law in the event of a consumer suffering an adverse reaction is much more complicated. Not least, a manufacturer's liability may be influenced by the publicity previously given to the particular allergen, the extent of any precautions he may - or may not - have taken, and the way in which he may have alerted consumers to any potential risks. His liability may also be influenced by any steps that other manufacturers may have taken.

Current EU food labelling legislation generally requires ingredients knowingly added to a food to be declared clearly and legibly on the product label or pack. There are a number of exemptions from this basic requirement and, of course, not all food is sold pre-packed. However, proposed amendments to the EU labelling regime are currently being considered in the European Parliament. These will, if agreed, remove the exemptions for a defined list of the principal serious allergens and result in the known presence of such allergens being declared. However, of particular concern is that neither the existing legislation nor the new proposals are applicable to so-called 'adventitious' presence - those materials that might be present in the product - despite every effort that a manufacturer has taken to exclude them.

The potential implications of the new, additional labelling legislation on the requirements of other non-food legislation will need to be considered. Although the debate has largely been driven by adverse reactions to peanuts and tree nuts, there is now the need to consider whether similar precautions will in future need to be taken in respect of other life-threatening allergens - and, if so, how? It can be argued that the unavoidable consequence of the new statutory requirement to declare the known presence of an extended list of serious allergens will be an increase in the number of materials that become subject to the so-called 'defensive labelling' route of advising consumers of the possible, yet unavoidable, presence of these allergens in a wider range of products than currently.

Without doubt, most responsible food manufacturers and retailers are very keen to assist with this issue. The biggest problem is how best to discharge our legal responsibilities in a helpful, pragmatic and meaningful way. Some have questioned whether the label the

appropriate way to convey such messages to allergic individuals or whether alternatives such as databases are more appropriate. Most in industry believe very strongly that the product label is the only way to address the issue on a reliable, specific product-by-product basis.

The commercial reality of interchangeability of production sites (and even countries), the implications of introducing new products alongside existing ones, range and brand extensions (taking a well known brand into other food sectors), and the fact that household-name brands may therefore be 'safe' in one country but at risk in others lead us to conclude that a third party database route is unacceptable.

Accepting that labelling is the answer, the necessary prominence of the information then becomes a subject of much debate. Equally, if such information is considered 'essential' for pre-packaged foods, then it is necessary to consider whether, and to what extent, the same applies for all foods when sold loose, whether at retail or catering outlets, and in particular when identical products may be sold alongside each other - one labelled with full ingredients, the other not.

The importance of a food product database on allergens for consumers and industry

M. van Dusseldorp

TNO Nutrition and Food Research, the Netherlands

Nowadays, consumers request reliable information about the food they purchase, especially about their composition. Information is particularly important for those consumers who suffer from allergies or adverse reactions to certain substances. They need detailed and comprehensible information on the presence or absence of allergenic substances in food. By participating in the *Allergen Databank* (ALBA) the food industry makes this information available to consumers. Thus, ALBA is an important intermediary between producers and consumers.

The ALBA databank

The databank ALBA exploits an automated database containing data on food constituents that may give rise to hypersensitivity reactions. These data are based on information provided voluntarily by the food industry corroborated by recent scientific and technological developments. Emphasis is given to food components acknowledged as being responsible for food intolerance or food allergy. The data are being updated on a continuing basis. The information is used for compiling lists of branded articles. These lists or booklets are enumerates of products free from the substances eliciting the allergic response.

For inclusion of a component in the databank the following criteria are being used:

- medical personnel observe a relation with food hypersensitivity;
- scientific data support a relation with food hypersensitivity;
- information can not easily be traced from the food label; and
- manufacturers and suppliers have information available.

Currently (2002), the databank contains information on 33 components and 17000 products. The topicality of information is essential because the nature and the composition of food products are continually subject to change. ALBA takes care to present the information in a format comprehensible for both producers and consumers. The information is provided to consumers through the Netherlands Nutrition Centre. The costs of the databank are covered by grants from the Ministry of Health, Welfare and Sport and the sale of lists of branded food articles.

Importance of the databank to consumers

Much information on food composition is already available through the food label, but this information is not sufficient for allergic patients. The databank provides more specific and detailed information on:

- ingredients in products that constitute less than 25% of composed products;
- ingredients that are labelled using their category name;
- ingredients of which the source is not specified on the label;
- technological fillers, carriers or auxiliary substances;
- combinations of specified allergens in products; and
- presence of allergens due to carry-over (if available).

Importance of the databank to producers

The principle tool available for the food industry to help in the management of the risk of food-allergic reactions is to indicate clearly the composition of their products. Individuals sensitive to a particular food or ingredient can then avoid consuming the product in question. Through participation in an allergen databank like ALBA, producers can outsource this activity, and make use of the scientific knowledge and expertise on allergy and food composition at TNO. In the mean time, a proliferation of lists containing potential food allergens among different companies is being avoided.

Future perspectives

Mandatory food labelling

The proposal for an amendment to the food labelling Directive 200/13/EC [Brussels, 06.09.2001, COM (2001) 433 def.] urges the Allergen Databank to envisage her objectives. Ten major food allergens or group of allergens are proposed for mandatory food labelling. However, an allergen databank will still be needed to fill the gap of information on other substances that may cause hypersensitivity, and to provide information on the use of auxiliary substances and carriers. Furthermore, concerning the ten major allergens, the databank can provide consumers with more specific information. For example, in ALBA refined oils of soy, peanut, nut and sesame are coded separately from cold pressed oil and protein. The reason is that refined oils hardly contain any protein. Those patients that exhibit fierce reactions to even minute amounts will avoid both the protein and the refined oil. Those who are less sensitive, have an appreciable broader choice of products in the lists of permitted branded products by splitting up the nuts, soy, peanuts and sesame and their refined oils. Due to such specifications, 17 substances in ALBA correspond with the 10 major food allergens for mandatory labelling. Also, it is not yet clear in what cases exceptions will be made to the proposed 0% rule that will replace the current 25% rule. For patients that react to minute amounts, a 2 or 5% rule will not be acceptable and thus will need more information than is provided on the food label. In all cases consumers like to receive information in a comprehensible and practical way.

Contamination

Through contamination, cross-contamination or carry-over allergens may be present in food products without knowledge of the consumer or even the producer. To be on the safe side, some producers classify their products as containing a specified allergen, even though it is no ingredient and the chance of contamination is negligible. Consequently, lists of branded articles free of specified allergens become shorter and shorter. Within ALBA the classification of either (+), (-), or (?) needs refinement in the near future. The fact that the problem of contamination is not being tackled by the proposed mandatory food labelling makes a refinement of classification even more urgent. One possibility is to define the (+) as 'present as ingredient or through carry-over', the (-) as 'not present guaranteed by HACCP', and split the (?) into two categories with different chances of contamination (e.g. contamination is highly unlikely as allergen is extraneous to process, product and location, versus contamination may occur). With such a system, consumers for whom contamination is not a serious threat have a considerable broader choice of products. Furthermore, information on threshold levels will mean a great step forwards and will give new opportunities for registration of allergens and selection of products, but here we still have a long way to go.

Internet

The ALBA just received a grant from the Ministry of Health, Welfare and Sport to realise data exchange and data communication through Internet. This development will facilitate and fasten data communication between ALBA, producers and consumers concerning the

presence and absence of allergens in food products. Furthermore, this initiative clears the way for advanced electronic food information systems on allergens in food, but also on other substances in food or information on production processes. Of course, the confidentiality of information concerning product composition and processing needs to be guaranteed.

Do quality systems guarantee safe food for allergic persons?

H. Byrnes

CIES, France

In April 2000, a group of international retailer CEOs identified the need to enhance food safety, ensure consumer protection, strengthen consumer confidence, to set requirements for food safety schemes and to improve cost efficiency throughout the food supply chain. Following their lead, the Global Food Safety Initiative (GFSI) was launched in May 2000. The Initiative is facilitated by CIES - the Food Business Forum in co-operation with the Food Marketing Institute (FMI). It is based on the principle that food safety is a non-competitive issue, as any potential problem arising may cause repercussions in the whole sector.

The key priorities of the Initiative are:

- to implement a scheme to benchmark food safety standards world-wide;
- to build and implement an international early warning system;
- to encourage co-operation between the world-wide food sector and national and pan-national governments and authorities; and
- to communicate the Initiative to all concerned parties and promote consumer education.

An international Task Force was formed soon after the launch of the Initiative to work on these priorities. It has since doubled in size and is now comprised of over 40 retailer quality managers whose companies represent 65% of food retail revenue world-wide.

The Task Force initially compiled a set of 'Key Elements' to serve as the requirements against which existing food safety standards will be benchmarked. The 'Key Elements' as defined by the Task Force are:

- Food Safety Management Systems;
- Good Practices for Agriculture, Manufacturing and Distribution; and
- HACCP (Hazard Analysis and Critical Control Points).

To make this set of requirements, a study was made of the Codex Alimentarius, legislative requirements, ISO standards and related Codes of Practice, taking into account a background of recent consumer health and safety concerns. Compliance with all components of the 'Key Elements' will lead to the endorsement of a submitted standard through the Initiative framework and subsequent acceptance by retailers.

The food safety standards that are endorsed can then be applied by food suppliers throughout the whole supply chain. The application of the endorsed standards to particular products will be at the discretion of retailers and suppliers. This process will vary in different parts of the world, depending on:

- company policies;
- general regulatory requirements; and
- product liability and due diligence regulations.

An outline of the Key Elements, requirements and a guidance document for certification bodies, and the endorsement procedure with a logo usage guide, are all contained in the 'Global Food Safety Initiative Guidance Document'. The use of the logo is clearly defined in the Guidance Document; it is not intended to be used on products, but will be used exclusively in business-to-business communication. The logo will mainly be used on official documentation to prove that the supplier is in compliance with an endorsed food safety

standard, and not on the products themselves. A draft version of the Guidance Document ('first edition') was widely circulated for external consultation between August 2001 and January 2002. External comments have been incorporated in the document and the second edition is now available. Copies can be downloaded from the CIES website www.ciesnet.com. Now that this version of the Guidance Document has been approved following the external consultation process, the second phase of the standardisation project will see the appropriate organisational structure taking shape. At the same time the formal endorsement process will be implemented. The system should be up and running end of spring 2002.

The Guidance Document in itself is not a food safety standard, but the requirements for food safety standards do contain certain criteria related to allergens. These can be found in the Good practices section of the Key Elements. The main requirement is:

6.2.10 Segregation and Cross-contamination

Procedures shall be in place to prevent contamination and cross-contamination of raw materials, packaging and finished product (GAP/GMP/GDP).

This then is further elaborated in Annex 1, which is an example of how a food safety standard should set its requirements:

Cross Contamination Risks

GAP

- *Cross-contamination by extraneous packaging should be avoided.*

GMP

- *There should be separation of raw and cooked products and utensils in high/low risk production areas.*
- *Nuts and other allergens should be identified and controlled to prevent cross contamination.*
- *Rework should be controlled.*

GDP

- *Not applicable.*

Segregation

GAP

- *Products types should be segregated to avoid cross contamination risks*

GMP

- *Products types should be segregated to avoid cross contamination risks*
- *There should be quarantine area for all reject/on hold products*

GDP

- *Products types should be segregated to avoid cross contamination risks*
- *There should be quarantine area for all reject/on hold products."*

In this example the requirements for Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP) and Good Distribution Practices (GDP) are set, so to cover the whole food supply chain from farm to fork. It is felt by retailers, that these requirements in addition to legal requirements are necessary to manage the ability to best sell food products to people with allergies, as this will allow for clear labelling of all products. The labelling of food products of the 'may contain' type should be avoided as much as possible as this unnecessarily limits the consumer with special needs in his choice.

The other priorities of the Global Food Safety Initiative are:

- Co-operation between the world-wide food sector and national and pan-national governments and authorities

Asking for a certificate showing compliance with an endorsed food safety standard is a preventative measure to minimise risk. This process will only begin to provide the food chain with guaranteed food safety. Food safety should be considered as a total food

chain responsibility within a legal framework, and a fully committed and responsible effort will be required from all stakeholders in the chain. Co-operation within the world-wide food sector and with national and pan-national governments and other relevant authorities is essential and will be actively encouraged, promoted and developed.

- **Early Warning Systems**

When a food safety issue arises, it is essential that information is made available and distributed quickly, accurately and clearly to all parties concerned. To address this need, an early warning system is being developed for the food industry, in close co-operation with suppliers. The objective is to provide a mechanism for the exchange of both general and crisis-related information, in harmonisation with existing legal and governmental frameworks. It will be put into operation later this year.

- **Consumer awareness**

A consumer food safety awareness campaign will be developed at a later stage.

Increasing awareness of food allergy

F. Timmermans

European Federation of Asthma and Allergy Associations (EFA), the Netherlands

I would like to express my gratitude for allowing a representative of the European Federation of Asthma and Allergy Associations to speak at this Forum. As a member of the Dutch Food Allergy Association and the Dutch Anaphylaxis Network I am here to give some insights on increasing awareness of Food Allergy from a patient point of view.

First I would like to start with some background information on the rise of a food allergy patient organisation in the Netherlands. It must be the spirit of time because in the UK and the USA the same movement happened at about the same time. Back in the eighties of the last century food allergy was no topic in the public mind. Of course there were people who suffered from food allergies, but in general there was no attention, no understanding and no help. Although there was some concern about food safety and a rise in awareness on the quality of biological grown foods, the major part of the public had no idea of food allergy or food intolerance at all. For the Forum's sake we address our attention on food allergy and not on food intolerance because from a scientific point of view food allergy can be proven.

In the Netherlands, in 1985 the Dutch Food Allergy Association was founded by two mothers standing with the back against the wall in their search for recognition and treatment for this illness. In 1991 a National Food Hypersensitivity Information Centre was founded by this organisation and two other organisations, i.e. the Government Information Bureau for Nutrition and ALBA, the organisation controlling the database for adverse reactions to food. At this moment the Dutch Food Allergy Association has about 3500 participants, supported by an advisory board consisting of medical specialists, food technicians, dieticians and legal advisors. Being a patient organisation, there are also approx. 60 national contacts, most of them with support groups. For special topics/goals there are six working groups within the organisation to address the specific support and need related to these goals. For instance, the Dutch Anaphylaxis Network is such a working group focussed on all aspects due to anaphylaxis. In the Netherlands this last group consists of approx. 200 families, probably being only a small tip of the iceberg.

So what has this done to the increase of awareness of food allergy in the Netherlands till now? The efforts of the Food Allergy Association gave rise to the awareness within the general public. Attention on this topic was given through newspapers, television and radio. Although we do not know for certain what this has done to the awareness of food allergy, the rise of telephone calls indicates an increase. And although there are a lot of people working on the item, there is a lot of work to be done.

Today we are gathered here, mainly by professional drive: scientific, medical, industrial and some of you by personal drive coming from patient organisations. But with all due respect to this Forum, the attention on food allergy mainly focuses on technological and medical research and solutions on, for instance, how to prevent an allergen being present in the end-product or, when this cannot be achieved, how to inform the consumer on the probable presence of an allergen. But do not get me wrong, these activities are very important for the safety of the allergic consumer. They must rely on the safety of the goods they buy. Or at least take their own responsibility in buying safe foods. Furthermore it is also

important that GP's and primary care institutions know about food allergies and what impact, physically and not in the least socially, it has on the patient and his or her family. Too often we come across children who want to attend school, kindergarten or children's day care, but are refused for a being a child at risk of some severe reaction to food. Somehow this rises fear amongst those who should care for the child and gives grief and anger amongst those caring for the child.

So what has to be done to increase awareness of food allergy in such a way that it is no threat to the patient and the society? Let me state that I do not have the solution for this problem, but I have some ideas which I want to share with you.

Address the problem

I think it should be made clear that food allergy is a growing serious concern in public health. At least it is an annoyance and sometimes it can be life threatening. But it always has an impact on someone's life. So food allergy is not only a medical problem, but it is a social problem as well and therefore might be translated into an economic problem. Food is everywhere and is not only used for eating, but increasingly used for other purposes such as the production of cosmetics and medication.

Communicate

Because I have a child at risk of anaphylaxis I know there is more going on in research on food allergy than the average consumer. I look on labels, compare products, I call manufacturers, look for additional information, etc. But even if all the researchers are working on solutions for food allergy topics, we - the public, the patients - will not know what is going on. Unless we are told what is going on.

Co-operate

There can only be a win-win situation if the parties concerned co-operate on mutual items. We must not be negligent about the value of patient input. There are patient organisations that are willing to co-operate and to find solutions for the rising problems.

Educate

Anyway, even though we succeed in increasing awareness of food allergy within the public, they will come across ignorant caregivers who will not believe that food can give such adverse reactions that people cannot function anymore. Did you know that in the 4-year basic doctor's course only about 8 to 12 hours is spent on food? And of those hours only a small percentage is spent on food allergies. So you cannot blame them for not knowing. Perhaps praise the ones who know only from their own interest. We have to educate our doctors, not only them, but nurses and other caregivers too. Start at the basis for starting medical students together with extra courses for the GP's and specialists. From there we can spread the word.

Accept

We also have to inform the public on this illness - although I do not call it an illness, but a disease - because if an allergic person does not come into contact with the allergen, he or she will not get ill. I think this point has to be stressed too. One does not get ill, when no allergen occurs. And this starting point is the key to a normal life for all allergic people. It must be and can be organised that people who are allergic, can live a normal life. Help them

to take their own responsibility, however, society has to facilitate that. Accept that there are people who have a disease, but do not have to get ill. They will not charge you for participating and helping them. It should not have to be a question from them but one from us. Just two simple questions for instance at schools: 'are you allergic?' and 'what can we do to help?'

Organise

Of course the work you all are doing is of eminent importance for the basis of a safe life of allergic people and you all have to keep on working for that sake. But perhaps we can also start today by looking over the borders of our own field and realise that there is a world out there which needs to be informed. I know it will not be easy to do, but if we will we can. And I know there will be a lot of allergic people who will say: thank you!

Looking into the future: scientific and regulatory perspectives

K. Vierk and K.J. Falci

Food and Drug Administration, USA

There are many sincere efforts undertaken by the United States food industry and the United States Food and Drug Administration (FDA) to control the unwanted presence of food allergens in food products. Whether it is due to the sheer volume of food products produced for sale in any given year or to errors in judgement on a food processing line, recalls of food products containing undeclared allergens still occur. Additionally, exemptions in the United States food labelling laws, such as collective naming and incidental ingredients for the naming of ingredients in the food ingredient statement, also help to add confusion as to when an allergenic ingredient should be labelled in the ingredient statement. In April of 2001 the agency issued a Compliance Policy Guide for food allergen labelling and for the prevention of cross-contamination by food allergens in food processing plants. This year the FDA is stepping up its search for undeclared allergens in food products by training our FDA field investigators on how to inspect firms producing food products susceptible to contamination with allergenic ingredients. In addition, as part of current food allergen inspections, a questionnaire concerned with industry good manufacturing practices related to allergens will be discussed. Finally, the presentation will address the 2002 priorities of the FDA on food allergens, allergen thresholds, the latest thinking on food allergen detection, and the FDA's recalls of foods due to undeclared allergens.

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¹Florida State University, USA, ²US Department of Agriculture, USA and ³University of California, USA
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¹University of California, USA and ²Florida State University, USA
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¹University Medical Centre, the Netherlands, ²CLB, the Netherlands and ³University of Nebraska, USA
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¹University Medical Center, the Netherlands, ²TNO Nutrition and Food Research, the Netherlands and ³University of Nebraska, USA and ⁴CLB/Sanquin, the Netherlands

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M. Wensing¹, A.H. Penninks², S. Hefle³, S.J. Koppelman², C.A.F.M. Bruijnzeel-Koomen¹ and A.C. Knulst¹
¹University Medical Centre, the Netherlands, ²TNO Nutrition and Food Research, the Netherlands and ³University of Nebraska, USA

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A. van Amerongen¹, S. Peres¹, M. Appeldoorn¹, M. Koets¹, L. Zuidmeer², R. van Ree² and H. Wichers¹
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¹Plant Research International, the Netherlands, ²Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, the Netherlands and ³University Medical Centre, the Netherlands
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¹Experimental Nursery of Shanxi Forestry Department, China, ²Plant Research International, the Netherlands and ³University of Vienna, Austria

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¹Plant Research International, the Netherlands, ²Leiden University Medical Center, the Netherlands, ³Wageningen Centre for Food Sciences, the Netherlands and ⁴Rijnstate Hospital, the Netherlands
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L.J.W.J. Gilissen¹, A. A.C.M. Peijnenburg², H.P.J.M. Noteborn², H.J. Wichers³ and G. Beers⁴
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¹TNO Nutrition and Food Research, the Netherlands and ²University d'Auvergne, France
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Multi-screening immunoassay for the detection of nut proteins in chocolate

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Health Canada, Canada

Testing for the presence of potential allergens in processed foods is part of the quality control process for some commodities where cross-contact is possible. It is also an important task in the surveillance protocol of laboratories affiliated to regulatory institutions in charge of enforcing the constantly evolving regulation for food ingredient labelling in relation to allergens. Screening systems dedicated to specifically identify one of the potential food allergens, may be of great usefulness if they are performed as part of a multi-ingredient screening system, where a single sample run allows the determination of the incriminated undeclared ingredient.

A multi-nut enzyme immunoassay was developed to check for the presence of peanut, hazelnut, almond, cashew and brazil nuts in one single run. The assay was designed under the competitive indirect format and was adapted for screening purposes applied to chocolate samples. The cut off level detected with this assay was 2 ppm of protein for each ingredient.

In most cases, the high specificity of the used antibodies allowed the identification of each particular allergen with no possible confusion. This assay was proven to be useful as part of an analytical procedure involving the identification of the unknown allergen in recalled samples prior to the application of the quantitative technique to determine the level of cross-contamination.

Such assay was also designed to be a semi-quantitative indicator for each ingredient. Calibrators such as 1, 5 and 10 ppm may be included in the assay format to allow a better appreciation of the level of protein present in unknown samples.

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Optimised immunoassay for the detection of hazelnut in chocolate

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A competitive enzyme linked immunosorbent assay (ELISA) was developed to detect hazelnut using polyclonal antibodies generated against a protein extract of hazelnut. No cross-reactivity was observed when tested against 39 commodities, including many common other allergens. Standards at a spiking level lower than 0.45 ng/ml of proteins were clearly identified by the ELISA (IC80 of the competitive test). An extraction and quantification method was developed and optimised for chocolate, the major food commodity likely to be cross-contaminated with undeclared hazelnut during food processing. No sample clean-up was required when extracts were diluted 10 fold, overcoming any potential matrix effect. Hazelnut was recovered at 63.8-83.4% when a blank chocolate was spiked at levels varying from 1 to 10 ppm. A confirmation technique was developed using SDS-PAGE electrophoresis and western transfer. The developed methods were shown to be efficient when applied to a small market survey, which showed the presence of undeclared amounts of hazelnut in some chocolate products.

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On the use of chicken immunoglobulins for the detection of peanut proteins in foodstuffs

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Peanut proteins are one of the most important food allergens described. It is expected that the number of incidences will increase in the years to come, partially because of a growing exposure to these proteins. The symptoms of the allergy, which can be induced by very low amounts of allergen, may vary from rather mild to very severe, including death due to anaphylactic shock. Efforts to guarantee better food safety will probably result in a more detailed regulation with regard to labelling. Consequently the food industry is confronted with the need for analytical tools, such as immunochemical methods, to confirm the absence of allergens. Enzyme linked immunosorbent assays (ELISA), using monoclonal or polyclonal mammalian antibodies are currently available for the detection of peanut proteins. In the current research, the usefulness of chicken immunoglobulins to detect peanut proteins in foods is investigated. Chickens have a large number of advantages compared to mammals for antibody production. Despite the ease of chicken immunoglobulin production and isolation, their use of the detection of peanut protein in ELISA's is not yet described.

Peanut proteins, extracted from raw and roasted peanuts, were used to immunise chicken hens. Immunoglobulins were isolated from the egg yolk using an aqueous dilution technique. Injection with proteins from roasted peanuts resulted in the isolation of antibodies with higher titres compared to the immunoglobulins obtained from the hens immunised with proteins from raw peanuts. The isolated immunoglobulins were used in an indirect ELISA in order to evaluate their applicability for the detection of peanut protein in aqueous solutions. Again the antibodies obtained by injection with roasted peanut proteins proved to be better because of the higher sensitivity of the assay. Assay sensitivity was not highly influenced by the characteristics of the competition buffer used, which was in contrast to an earlier immunosorbent assay, using chicken antibodies, developed in our laboratories. Both assays (for raw and roasted peanut), proved to be very specific: no significant cross reactivity was observed towards proteins isolated from other legumes and nuts or towards other dominant proteins present in foods. Preliminary experiments for the detection of peanut protein in real food matrices illustrate the high potential of the developed assay.

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P4

Sensitive and specific biosensor detection of hazelnut proteins and other allergens in food

H. Jonsson

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Trace levels of 'hidden' allergens in food can cause severe allergic reactions, including life-threatening anaphylaxis. Sensitive and specific analysis of such substances is therefore of major importance in food quality control. Currently, immunoassays such as ELISA and rocket immunoelectrophoresis are used to detect allergens in food matrices. However, these methods are labour-intensive, relatively slow and require high technical skills, making them awkward to use in routine analysis such as on-site food production control. As an alternative, a highly automated and rapid immunoassay based on an optical biosensor (BIACORE Q) was developed.

Polyclonal antibodies raised against hazelnut proteins, ovomucoid (egg) and beta-lactoglobulin (milk) were immobilised on replaceable sensor chip surfaces. When food sample extracts were injected over the surface, the amount of allergen binding to the antibodies was read out as a shift in the sensor response. Standard curves ranging from 0.005 to 10 $\mu\text{g ml}^{-1}$ were successfully established for pure extracts of the allergens.

To increase the specificity and to allow analysis of crude extracts, the extraction and analysis conditions were optimised for the detection of hazelnut in chocolate samples. Several approaches to reduce non-specific binding were demonstrated, including raise of pH and ion strength and use of a sensor chip with thinner immobilisation layer. Addition of polyvinylpyrrolidone decreased the non-specific binding and increased the recovery of hazelnut proteins in dark chocolate samples. With the use of a secondary antibody enhancement strategy, a sensitive and specific hazelnut 'sandwich' assay was realised.

Comparison of three commercial ELISA kits for the detection of peanut allergens in foods

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Background. Although peanut is a common source of protein, it can also cause allergic reactions ranging from mild skin rashes to severe anaphylactic shock, which, in rare cases, results in death. Minute amounts (in the ppm range or even lower) of peanut are capable of inducing such reactions. The detectability of peanut traces in foods is important to control compliance to existing and future labelling regulations - as peanuts can be processed to a variety of products, which are used as ingredients or as substitutes (e.g. for almond protein) - and to detect any unintentional contamination of foods, which do usually not contain peanuts (e.g. via processing lines, transportation containers, etc.). Recently several enzyme linked immunosorbent assays (ELISA) for rapid and highly specific detection of peanut protein were placed on the market. The aim of our study was to compare the performance of three commercially available peanut ELISA test kits and to investigate the effect of various roasting conditions on the detectability and the quantification of peanut products in a matrix like corn flour.

Methods. Peanut Protein Test Kit from Tepnel BioSystems (UK), Ridascreen Peanut from R-Biopharm (Germany) and Neogen Veratox Peanut Protein Test Kit from Biological Analysis Systems (Germany) were employed to detect peanut in corn flour in the low ppm range. The detection limit, recovery, precision, accuracy and repeatability of the different Elisa kits were investigated. To achieve this, defatted fine ground peanuts, raw or dry roasted at 110 °C for 1, 2, 3, 4, and 5 hours, respectively, were homogenised in an extraction buffer using an Ultra Turrax and aliquots were spiked into 1 to 4 grams of corn flour to yield concentrations between 0.1 and 20 ppm. Finally samples were extracted and the respective peanut content was determined by following the manufacturer's instructions.

Results. All of the kits tested were able to quantify peanut traces in the very low ppm range with detection limits between 0.25 and 0.5 ppm. The kits' precision, accuracy, and repeatability were satisfactory. Background signals from the corn flour matrix were minimal. In the range between 5 and 10 ppm the kits slightly over-estimated the actual peanut content. At the very low ppm range over- or under-estimation of the peanut content was rather dependent on the kit employed. Generally raw peanuts gave higher results than roasted peanuts at the same concentration. After prolonged roasting times (≥ 2 hours) the recovery of peanut protein decreased significantly.

Conclusion. The peanut ELISA kits investigated were equally applicable to qualitatively detect peanut traces in foods. However, quantification characteristics differed significantly between kits, especially at the very low ppm range and close to the detection limit. These findings might become an important issue in the discussion of legal threshold levels for allergen 'contaminants' in foods declared to be 'peanut free'. In our opinion it is currently very unlikely to analytically quantify the precise peanut content in foods by employing commercial ELISA kits, as the supplied standards do not comply completely with the tested allergen in the food matrix (different species, various processing procedures, etc.), and hence also differing antigens could have been used for antibody production. In addition, significant amounts of peanut protein might not be detected after harsh heat treatment while at the same time retaining their allergenic potency.

Biochemical characterisation of almond (*Prunus dulcis* L.) major storage protein amandin*

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Human sera IgE from almond allergic patients recognise amandin, the major storage protein in almonds. Amandin was prepared by column chromatography (amandin-1), cryoprecipitation (amandin-2), and isoelectric precipitation (amandin-3) methods. Regression analysis of the ultracentrifuge data as a function of protein concentration yielded $s_{20,w}^0 = 14.10 - 0.16c$ where c = protein concentration in g/100 ml (range 0.2-0.9% on a dry weight basis). Amandin is therefore a 'legumin' type protein characterised by a sedimentation value of 14S. Isoelectric focusing of amandins indicated a pI range of 5.9-7.2. Amandin is composed of two major types of polypeptides with estimated molecular weights 42-46 kDa (acidic polypeptides) and 20-22 kDa (basic polypeptides) linked via disulfide bonds and several additional minor polypeptides. The estimated molecular weight of amandin is 427,300 Da \pm 47,600 ($n = 7$). Amandin has a Stokes' radius of $65.88 \pm 3.21 \text{ \AA}$ ($n = 7$). Con-A Sepharose column chromatography as well as glycoprotein staining of SDS-PAGE gels indicated that amandin is not a glycoprotein. ELISA assays and Western blotting studies indicate amandin-1, amandin-2, and amandin-3 are antigenically related. Heat denaturation did not reduce antigenicity of amandin. Reduced and carboxymethylated amandin was subjected to reverse phase (C₄) HPLC and the separated polypeptides A1, A2, B1, B2, B3, B4, B5, and B6 were subjected to N-terminal amino acid analyses. N-terminal sequences for A1, B1, and B3 were respectively RQSQLS, RQSQLSPQNQC, and GVEETFCSARLSQN. These N-terminal sequences had 100% identity with prunin precursor cDNA derived amino acid sequence (Pru2 protein precursor, Pfam PF00190, EMBL X78120.1), for a hexameric 11S (legumin family) seed storage protein from almond (*Prunus amygdalus* Batsch) seeds. Methionine is the first essential limiting amino acid in amandin followed by lysine and threonine.

* Funded in part by the College of Human Sciences (CHS research Initiation Awards Program) and by the Council for Faculty Research (COFRs), Florida State University, Tallahassee, FL, Almond Board of California, and the USDA NRICGP (#990 1530).

**Ana o 1, a major cashew nut (*Anacardium occidentale* L.)
allergen of the vicilin family***

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The allergens responsible for cashew food allergy have not been well characterised. We have screened a cashew cDNA library with human IgE and rabbit IgG anti-cashew extract antisera, sequenced the identified clones and expressed a recombinant allergen in *E. coli*. Immunoblotting was used to screen for reactivity with patient sera and inhibition of immunoblotting was used to identify the native peptide in cashew extract. Fifty plaques reactive with both human and rabbit antisera were identified and four clones were sequenced. The four were closely related in sequence to one another, sharing a common core sequence. A screening of the remaining unsequenced clones by PCR indicated that 45 of 46 were also similar or identical in sequence to Ana o 1. A search of the gene data bank showed that these sequences encode members of the vicilin/sucrose binding protein family of plant proteins. Screening of sera from patients having experienced allergic reactions to cashews with the recombinant fusion protein showed 10 of 20 (50%) reacted with the recombinant protein. Inhibition of the reactions showed the corresponding native allergen protein, designated Ana o 1, to be ~50 kDa. In conclusion, this vicilin-like protein is a major allergen in cashew nuts.

* Funded in part by the College of Human Sciences (CHS Research Initiation Awards Program) and by the Council for Faculty Research (COFRs), Florida State University, Tallahassee, FL.

Allergen recognition patterns in a Dutch hazelnut allergic population in relation to individual reactivity patterns as observed during double-blind placebo-controlled food challenges*

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Background. Hazelnut allergy is common in Western and Northern Europe. Sensitisation to hazelnuts in these areas can mostly be attributed to cross reactive IgE directed to a protein in hazelnut, Cor a 1, with high homology to the major birch pollen allergen Bet v 1. Other (non pollen related) hazelnut allergens like LTPs and 2S albumins have been described and are thought to be associated with more severe symptoms.

Objective. To reveal allergen recognition patterns in a Dutch hazelnut allergic population (n=29) and to correlate these results to the individual clinical reactivity patterns.

Methods. Sera were obtained of 29 patients, who participated in a previous study, with positive DBPCFCs to hazelnut. Minimum provoking doses (MPD) varied from ≤ 1 mg up to a dose between 30 and 100 mg hazelnut protein. Symptoms varied from OAS (n=29) and additional gastro-intestinal symptoms (n=5) to objective symptoms (n=2) of lip swelling and generalised urticaria. The presence of specific IgE to hazelnut was determined using CAP system FEIA (Pharmacia Upjohn) and radio allergosorbent tests (RAST). Immunoblotting was performed using raw hazelnut extract and pH2.5 hazelnut extract, mainly representing low molecular weight allergens (Akkerdaas, J. et al., Poster no.71 presented at the Symposium on Immunological, Chemical and Clinical Problems of Food Allergy, 11-13 March 2001, Venice).

Results. Specific IgE to hazelnut was found in all but two patients using RAST (> 0.3 IE/ml) in contrast to 17 out of 29 using CAP system FEIA (> 0.35 kU/ml). Immunoblotting using raw hazelnut extract demonstrated IgE binding to Cor a 1 (17 kDa) in 23 sera, of whom 3 sera had additional IgE-binding to a 30-32 kDa protein. In two sera exclusive IgE binding to high molecular weight proteins was demonstrated. Immunoblotting using pH2.5 hazelnut extract revealed additional IgE binding to a 6-8 kDa protein in 2 sera and to three bands of < 17 kDa in 1 serum. Four sera did not display any IgE binding (despite positive RAST and CAP in three of these sera). Subdividing patients on the basis of their allergen recognition pattern revealed 17 patients with exclusive Cor a 1 recognition and eight patients with (exclusively or additionally) recognition of other allergens. When comparing MPDs and observed symptoms as were observed during DBPCFCs of these two groups no significant differences could be revealed. However, in 5 out of 8 patients (63 %) with 'other' recognition patterns the challenge had to be discontinued prematurely because of longstanding subjective symptoms in contrast to 4 out of 18 Cor a 1-sensitized patients (22%). Besides, the only observed objective symptoms occurred in patients with no Cor a 1-recognition on immunoblots.

Conclusion. Most hazelnut allergic patients in this study are sensitised to Cor a 1. A minority of patients appeared to have IgE directed to proteins of 6-8, <17 or 30-32 kDa. Probably due to the low number of patients with other allergen recognition profiles, only slight, non-significant, differences could be revealed in MPDs and severity of symptoms among patients with different recognition profiles.

* This study was financially supported by ILSI.

Determination of threshold levels of patients with hazelnut allergy using double-blind placebo-controlled food challenges (DBPCFCs)

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Background. Because hazelnuts are used in pre-packaged foods, sometimes without clear notification on the label, accidental ingestion occurs. To evaluate the risks for hazelnut allergic patients after accidental ingestion of low quantities of hazelnut, it is important to reveal the distribution of threshold levels in hazelnut allergic patients.

Objective. The aim of this study is to determine the distribution of threshold levels in hazelnut allergic patients using DBPCFCs.

Patients. Adult patients with hazelnut allergy, as determined by a positive history of adverse reactions, positive skin prick test ($\geq 2+$) and/or elevated specific IgE-level (CAP \geq class II) were included in the study.

Methods. Challenges were performed using seven placebo doses and seven doses of roasted hazelnut, ranging from 30 μ g to 30 mg of hazelnut protein, hidden in mashed potatoes. The schedule of seven placebo and verum doses was random with an interval of 30 minutes when no reactions occurred. A challenge was discontinued when objective reactions were observed or subjective reactions lasted for longer than one hour. The challenges were conducted in a clinical research setting equipped for monitoring of vital signs and resuscitation. Ethical approval for this study was obtained from the hospitals ethics committee. All patients gave written informed consent.

Results. The first DBPCFCs of nine hazelnut allergic patients (4 women) were all negative. Since we were not sure whether the doses were too low or whether we should use raw instead of roasted hazelnuts, we investigated the heat lability of the 18 kD major hazelnut allergen, Cor a 1, that shows high homology to the major birch pollen allergen Bet v 1. Using a competitive RIA with monoclonal anti-Bet v 1, radiolabelled Bet v 1 and raw hazelnut meal (incubated with temperatures of 20 up to 100 °C) together with the roasted hazelnut meal, we demonstrated that binding of Cor a 1 was severely decreased after heating of 80 °C and higher. No activity of this allergen could be detected in the roasted hazelnut meal. Therefore, we decided to use raw hazelnut meal for the DBPCFCs with slightly higher doses (1mg - 1g). The results of six rechallenged allergic patients are summarised in the table below.

Reaction	Threshold dose							No reaction
	1 mg	3 mg	10 mg	30 mg	100 mg	300 mg	1 g	
Subjective (n)	1	1	0	2	2	0	0	0
Objective (n)	0	0	0	0	0	0	0	0

No objective reactions were observed. All subjective reactions consisted of itching of throat, tongue and/or lips and occurred within 2 to 10 minutes after ingestion of the test meal. Patients did not react to any placebo dose. Late reactions did not occur.

Conclusion. From these preliminary results it appears that threshold levels for subjective reactions in hazelnut allergic patients vary from less than 1mg up to doses probably higher than 100 mg of raw hazelnut protein. Another important result is the observed heat lability of the major hazelnut allergen, implicating that heating of hazelnuts decreases the allergenic potential for a substantial part of the hazelnut allergic population.

The distribution of individual threshold doses eliciting allergic reactions in a peanut allergic population

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Background. Peanut allergy is an important food allergy as is illustrated by its high prevalence and the association with severe allergic reactions. Thereby peanuts are widely consumed due to the ubiquitous presence of peanut in pre-packaged foods. Hidden peanuts can endanger peanut allergic patients. To assess the risks for a peanut allergic population of developing allergic reactions after accidental ingestion of peanut, threshold doses for eliciting allergic reactions need to be elucidated.

Objective. The main objective is to reveal the distribution of individual threshold doses in a peanut allergic population and secondly to correlate these threshold doses with the severity of peanut-induced symptoms.

Methods. Twenty-six adult patients with a convincing history of peanut-related symptoms, a specific IgE-level of ≥ 0.7 kU/L and/or a positive SPT of $\geq 2+$ to peanut were included. They underwent double-blind placebo-controlled food challenges (DBPCFCs) with increasing doses of peanut meal. The severity of peanut-induced reactions was correlated with individual threshold doses.

Results. All patients had a positive challenge and suffered from reactions of subjective oral symptoms (n=26), prior to gastro-intestinal (n=14) and/or objective symptoms (n=5). Objective reactions consisted of swelling of lip (n=3) and vomiting (n=2) accompanied by hoarseness, difficulty in swallowing and facial flushing in 1 patient. Reactions started within 30 minutes after ingestion of peanut except for 2 patients where additional symptoms were delayed by 1-2 hours. Threshold doses for allergic reactions ranged from 100 μ g up to 1 g of peanut protein. Patients with a history of severe symptoms had lower threshold doses compared to patients with mild symptoms ($p < 0.0001$).

Conclusions. Threshold doses in peanut allergic patients are low but vary widely. Fifty percent of a peanut allergic population (95% CI, 30-70 %) already will suffer from an allergic reaction after ingestion of 3 mg of peanut protein (about 1/50 peanut), requiring adequate declaration of (traces of) peanut in consumer products. This is even more important as patients, suffering from potentially severe reactions, have lower threshold doses.

The major apple allergen Mal d 1: localisation and influence of oxidation on its allergenicity

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In Europe, over one million people are suffering from apple allergy. This allergy, being part of the so-called Oral Allergy Syndrome, is represented by IgE-mediated symptoms occurring mainly at the mucosa of lips, tongue and throat after ingestion. In addition to direct consumption apple is used in a great number of products such as cider, apple juice, wine, liqueurs, and vinegar, as filling for cakes, pies and sauces. However, the consumption of fresh, unprocessed apples is the main cause of apple allergy, often resulting from a cross-reactivity to an initially developed allergy against the major birch pollen protein, Bet v 1. Indeed it is reported that approximately 65% of birch pollen allergic patients are sensitised to this fruit.

The major apple allergen, Mal d 1 (allergen 1 from *Malus domestica*), is an 18 kDa protein sharing over 60% amino acid sequence homology with Bet v 1. It has been demonstrated that Mal d 1 and Bet v 1 cross-react not only at the serological (IgE) but also at the cellular level (helper T-cells). Mal d 1 belongs to a group of plant proteins, the Bet v 1 family, showing significant sequence identity with pathogenesis related (PR) proteins of the PR-10 family. The function of these proteins is not known, but they are synthesised as part of a plant's response to stress and pathogen attack. As such they have been termed PR proteins. Apple also contains the Mal d 2 allergen, a thaumatin-like protein, the Mal d 3 allergen, a lipid transfer protein which is suggested to be cross-reactive to homologous proteins in peach and pear and the Mal d 4 allergen, cross-reactive with Bet v 2. Expression of Mal d 1 varies between different apple strains, Golden Delicious and Granny Smith having a high allergenic potency in comparison with other varieties. The allergenic potency is dependent on the ripening stage of the fruits, whereas it was shown that peels are more allergenic than pulps. It is well known, although not widely investigated, that allergens from fruits are generally labile and easily denatured by food processing and preparation. Cooking an apple will destroy its allergens, allowing sensitised persons to eat the food. Literature reports that it is sufficient to cut an apple and store it minutes to hours to largely decrease its allergenicity.

We have investigated the localisation of Mal d 1 in apple tissue to assess relative and absolute amounts of Mal d 1 in peel, pulp and core of three varieties. Furthermore, the influence of oxidation on Mal d 1 stability and/or allergenicity has been studied. Levels were determined in a competitive ELISA with Mal d 1, purified from apple by affinity chromatography, as the coating antigen. Standard curves were prepared with known amounts of the allergen and levels of Mal d 1 in apple tissue were expressed as IC₅₀ values calculated from the incubation of a dilution range for each sample.

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Genomics and proteomics for identification and quantification of allergenicity in apple cultivars

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Breeding for hypoallergenic apple cultivars requires a multidisciplinary approach involving expertise not only from plant breeders, but also from biochemists, molecular biologists, medical experts and allergologists. In a project, funded by the Ministry of Agriculture, Fisheries and Nature Management and by the EU, three Dutch research teams participate with the aim to develop hypoallergenic apple cultivars. The University Medical Centre Utrecht contributes with skin prick tests on volunteers aiming to identify the differences in allergenicity within a broad collection of apple cultivars. The Central Laboratory of the Netherlands Red Cross Blood Transfusion Services, Amsterdam, performs immuno-assays on allergenicity (IgE-binding), and purifies Mal d 1, the major apple allergen, from various apple cultivars. Plant Research International, Wageningen, supplies the apple cultivars and explores the plant material at the genomic and proteomic level, aiming at the development of molecular breeding markers.

At the protein level, Q-TOF MSMS is used as a novel and fast technique which directly provides information on the amino acid sequence and thus identifies the various isoforms of Mal d 1 in apple, also when these proteins occur as mixtures. In addition, Q-TOF MSMS allows quantitative measurement of the various isoforms. Thus Q-TOF MSMS enables to provide a complete, i.e. qualitative plus quantitative, picture of the presence of Mal d 1-proteins in fruits of different apple cultivars.

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Mapping four allergen genes of apple on a molecular linkage map

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Apple is a relevant source of food allergy for approx. 70% of tree pollen allergic patients (e.g. allergic to birch and hazel). One strategy aiming at reduction of apple allergy is breeding for low-allergenic apple cultivars. Breeding of apple is time consuming. Speeding up breeding for reduced allergenicity requires knowledge on the genomic localisation of the relevant genes, their expression pattern and their allergenic impact). In addition, allergenicity-related genetic markers are needed that enable to assess the allergenicity of a new apple cultivar already at the seedling stage. The current project (carried out within the EU-SAFE project of FP-5), focuses on the mapping of four apple allergen genes: Mal d 1 (Bet v 1 homologue; PR10 protein); Mal d 2 (thaumatin-like protein); Mal d 3 (lipid transfer protein) and Mal d 4 (profilin).

The mapping procedure included several steps:

- the design of DNA primers based on the occurrence of conserved areas in the allergen gene as published in public data bases
- optimising PCR conditions (especially to prevent artificial recombinant PCR product);
- identification of true polymorphisms and design of polymorphisms (markers) specific for the parental cultivars of segregating populations; and
- linkage of the allergen markers to known (i.e. mapped) markers in segregating offspring populations of three molecular linkage maps.

Using this procedure, four loci for Mal d 1 were identified, two on linkage group (chromosome) 13 and one on linkage group 16. On the basis of homology between the linkage groups 13 and 16, the fourth Mal d 1 locus is expected to exist on linkage group 16. Mal d 2 was found on linkage group 9, Mal d 3 on linkage group 4, and Mal d 4 on linkage groups 2, 8 and 9. Also for the latter three allergen genes, additional loci were identified, and their mapping positions are under study.

These results indicate that in a diploid heterozygous apple cultivar, for example, Mal d 1 can occur as eight different proteins. Questions now arise about the degree of expression in the apple fruits of the genes from the different loci, and on possible differences in the allergenicity of the individual gene product within and between cultivars.

Further strategies at Plant Research International aiming at reducing apple allergy include selection for low-allergenic apple cultivars from the wide variety of apple cultivars on the market, and specific knocking out the major apple allergen, Mal d 1, using antisense technology.

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For information on the EU-project SAFE QLK1-CT-2000-01394: www.akh-wien.ac.at/safe

Profiling gluten genes and proteins in wheat species and cultivars with respect to coeliac disease

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The position of wheat in the food industry is mainly due to the unique viscoelastic, gasholding and thermosetting properties of its major protein fraction: gluten. However, the importance of gluten is overruled by its characteristic to provoke coeliac disease (CD; gluten intolerance) in sensitive persons (~0.5 to 1% of the population) upon ingestion. CD-patients have to follow a lifelong strict gluten-free diet. The present joint research aims at identification and selection of non-toxic or low-toxic wheat cultivars to allow production of wheat products for CD patients and to help prevent the disease. The project includes:

- genomics of wheat gluten genes;
- epitope screening of a broad collection of wheat species and cultivars;
- clinical testing of selected cultivars with reduced toxicity;
- testing of industrial quality of proven low-toxic cultivars;
- introduction of suitable cultivars in the food production chain.

Hence, the project will contribute to a reduction of the coeliac problem world-wide.

The number of gluten genes can range from 25 to 150 genes, depending on the wheat species, its cultivar and the ploidy level. The gluten protein composition can differ considerably between species and cultivars, and can also vary within a cultivar due to growing-conditions. The start of the project focuses on screening and genomics characterisation of a representative selection of wheat species and cultivars from the wheat collection of the Centre for Genetic Resources, the Netherlands (www.genebank.nl). This selection includes *Triticum aestivum* (hexaploid, carrying the AABBDD genomes, old landraces and old and recent summer and winter cultivars), *T. turgidum* (tetraploid, with AABB genomes) and various (wild) diploid ancestor species (donors of the A, B or D genome). Using a specifically designed set of primers, the profile of the gluten gene family was analysed with focus on sequence composition and domain organisation. Concomitantly, gluten proteins have been extracted from grains for testing in a T-cell panel specifically sensitive to gluten epitopes considered relevant to CD. The poster presents the first results of the project.

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The effect of gluten peptides on intestinal gene expression

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Ingestion of wheat gluten may result in an intestinal disorder called celiac disease (CD). The disease is generally considered to represent a type IV hypersensitivity in which CD4⁺ T cells are involved. However, the precise mechanism underlying the damage of intestinal tissue by gluten has not yet been elucidated. The aim of our current work is to analyse whether epithelial cells in the intestine are directly affected by gluten molecules and, if so, to what extent the effects represent a stress response. For that purpose, differentiated and undifferentiated Caco-2 cells are exposed to peptic/tryptic digests of wheat gluten and analysed for gene expression using the DNA microarray technology. Upon exposure of the cells total RNAs are isolated, labelled with fluorescent dyes via reverse transcription, and hybridised to microarrays. These arrays are self-made and contain human cDNAs, which mainly are derived from cDNA libraries enriched for genes expressed in intestinal epithelial cells. In addition, the arrays contain cDNAs representing genes known to be involved in biotransformation and cellular processes such as proliferation and apoptosis. Preliminary results of these microarray hybridisations indicate that the expression level of particular genes has been changed after exposure of Caco-2 cells to a wheat gluten hydrolysate. It can be envisaged that the outcome of such experiments will contribute to a better insight in the molecular mechanisms underlying celiac disease and will result in the identification of biomarkers which can be used in *in vitro* bioassays for the screening of wheat cultivars and processing techniques for decreased gluten toxicity.

Quantitative determination of wheat germ agglutinin activity in wheat-based foods by enzyme-linked immunosorbent assay using glycoproteins immobilised on microtiter plates

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Plant lectins are carbohydrate-binding proteins able to affect various physiological functions, including immunological pathways. Most of these proteins are resistant to digestion in the GI tract and can affect the integrity of the intestinal epithelium and the absorption of dietary antigens. Moreover, lectins can also induce the release of allergic mediators from mast cells *in vitro*. Recently it has been indicated that wheat germ agglutinin (WGA) may affect the allergic response towards oral antigens in the gut-associated lymphoid tissue and that it stimulates IL-4 and IL-13 secretion by human basophils, indicating the potentiality to stimulate allergic responses to dietary antigens. However food thermal processing should have an effect on the biological activity of dietary lectins, thus affecting their influence on the immunological response to allergens.

It is therefore important to determine the activity (i. e. the sugar binding ability) of the lectins as they are found in processed food products. To this aim a new method has been developed, based on the immunological quantification of the lectin bound to specific glycoproteins immobilised on ELISA plates. The high detectability of the ELISA system allows to quantify nanogram quantities of active lectins. In this way WGA has been quantified in extracts of different wheat raw materials and heat-processed wheat foods. The obtained results demonstrated the presence of biologically active WGA in different foodstuffs, the amount of active WGA being related to the level of thermal treatment and to the presence of wholemeal flour or wheat germ. Active WGA was found to be absent in bread, cookies and crackers, whereas wheat germ-enriched pasta, wholemeal bread and wheat germ-enriched crispbreads contained 1410, 69 and 36 µg/100 g of active WGA, respectively. This indicates that the ingestion of at least some wheat-based foods can result in the possibility for WGA to act at the level of the GI tract, thus affecting the absorption of oral antigens and also their interactions with the immune system. Therefore, the involvement of WGA in the onset of food allergies *in vivo* seems to be a real possibility.

No difference in symptoms in milk intolerant adults during challenges with homogenised and unhomogenised milk

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It has been suggested that certain consumers tolerate untreated milk, but react to processed (i.e. homogenised and pasteurised) milk and dairy products. The reactions are proposed to be caused by different distribution of allergenic protein particles in fat droplets in homogenised milk compared to raw milk.

The aim of the study was to investigate the effect of homogenisation on the tolerance of milk in milk intolerant adults. Forty-eight subjects who had reported that they could consume unhomogenised milk without symptoms but experienced abdominal discomfort after consumption of homogenised milk, were challenged with two study milks for five days in a randomised, double-blind, cross-over study. The two milks studied were a) a homogenised, pasteurised commercial milk with fat content of 1.5% (Valio Ltd., Finland) with 20 ml whipping cream (fat content 38%) added per litre of milk for blinding, and b) an unhomogenised and pasteurised commercial organic milk with a fat content of 1.5% (Valio Ltd., Finland). During the study the subjects kept a daily diary of the consistency and number of their bowel movements and of any abdominal symptoms (abdominal pain, flatulence, bloating or nausea, scores 0-3 for each).

No differences in the symptoms during the challenges were found. Roughly half the subjects tolerated the homogenised milk better and the other half tolerated the unhomogenised milk better. During the milk-free period before the challenges the mean value for the sum of symptoms was 4.0 (SEM \pm 0.6). The mean symptom scores were 9.6 (\pm 1.3) for the homogenised milk and 8.4 (\pm 1.1) for the unhomogenised milk ($p=0.42$).

All the subjects experienced adverse gastrointestinal reactions during the milk challenges and therefore it is possible that some subjects may have had cow's milk protein allergy (CMA). Until recently CMA has been considered a disorder of early childhood (1), but lately, recovery from CMA has become a subject of controversy as some studies suggest that delayed non-IgE-mediated CMA might also be a disease of school-age children (2) and young adults (3, 4). Unfortunately, there is no reliable diagnostic method for determining delayed reactions to cow's milk protein, and therefore delayed CMA could not be diagnosed in the present study. Further studies are needed on the diagnosis, prevalence and treatment of non-IgE-mediated CMA.

The results of this study show no difference in the tolerance of homogenised and unhomogenised milk by the study subjects.

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Model of the development of the T cell response towards cow's milk in infants with and without cow's milk allergy

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Cow's milk allergy (CMA) in infancy is a transient disorder. More than 85% of the patients become spontaneously tolerant before the age of three years. Deviation of cow's milk (CM)-specific T cell reactivity is thought to play a central role in this process. We studied the T cell response in infants with CMA before and after development of tolerance, and compared this to infants without CMA. From these data, we propose a model for the development of the T cell response towards CM in atopic and non-atopic infants.

T cell clones (TCCs) were propagated from blood samples obtained from atopic infants with CMA (age 4-12 months) before (t=1) and after development of tolerance (t=2), from age-matched atopic infants without CMA at t=1 and t=2, and from age-matched non-atopic infants without CMA at t=1. After stimulation with CM, IL-4 and IFN- γ production was determined by ELISA, and expression of CD25 (activation marker) was measured by flow cytometry.

In infants with CMA, the T cell response is Th2-biased (increased IL-4). This fits with the current hypothesis that allergy is caused by a persistence of the fetal Th2 response. CD25 expression is increased, suggesting an intrinsically vigorous T cell response towards CM. In contrast, the response in infants without CMA but with atopy is Th1-skewed (increased IFN- γ), with low CD25 expression. This Th1-skewing may be required to induce a tolerant response towards CM, compensating for their atopic predisposition to develop a Th2-skewed response towards common allergens. In non-atopic infants, the T cell response to CM is Th0-like, with low CD25 expression. These infants develop tolerance to CM in a non-atopic milieu. Therefore, they may not need the strong Th1 bias, but can do with a moderate Th0 response. This is supported by the observation that, in atopic infants without CMA, the response at t=2 is shifted towards a moderate, Th0-like response, with low CD25 expression, which is comparable to the response in the non-atopic controls. Apparently, the tolerant response at long notice can be maintained by a Th0-like response with a low activation status. In infants with CMA that have become tolerant, the T cell response is deviated towards a more Th1-biased response, with low CD25 expression. This response seems comparable to that in atopic infants without CMA at t=1, and even tends towards the response in non-atopic infants at t=1. Therefore, it is likely that also in the infants who developed tolerance, the response will eventually become balanced, with moderate cytokine production and a low activation status.

This model provides an explanation for the differences in T cell reactivity that are seen between infants with and without CMA. These data, in addition to the observed deviation in T cell reactivity upon development of clinical tolerance, provide more insight into the natural course of the T cell response to CM in young infants with and without CMA.

Safety management of novel food production chains: on the assessment of the (potential) allergenicity of non-GM and GM novel foods

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There are several indications that the prevalence of allergy has increased during the last three decades. The reason is not fully clear, but explanations are sought in combinations of increased hygienic life style, including reduced contacts with microbes and helminths, and increased vaccinations and medication using antibiotics. Other reasons might be keeping breastfeeding from babies, and last but not least the changes in eating habits.

Current globalisation involves increasing imports of novel exotic food products into the Western market. Examples are mangistan (Malaysia), kumquat (Vietnam), kaki (China), sabra (Brazil), kiwi (New Zealand), Ngali-tree nut (Polynesia) and many others with sounding names. However, with the supply of such new foods, also new allergens have been introduced. One prominent example is the kiwi fruit (*Actinidia chinensis*) and its allergenicity to many Europeans and Americans. In addition, there is the potential of the introduction of allergens in novel food products through genetic modification (GM). Examples refer to the GM soybean containing Brazil-nut protein and the Starlink maize expressing the Bt-toxin. Introduction of new allergens into the Western diet should be prevented, especially towards children for whom allergies may have prolonged negative consequences with regard to the quality of their further life. Therefore, each novel food to be introduced into the European market, being non-GM or GM, should be tested according to EU regulation 258/97. Assessing the potential allergenicity of GM novel foods is relatively simple. The FAO/WHO (2001) developed a straightforward decision-tree for this purpose. However, the situation is much more complicated for non-GM novel foods.

The present project aims at the integration of two approaches. The first approach focuses on the insights and attitudes of the consumers towards novel foods, and on the effect of prior knowledge on e.g. the potential allergenicity (of non-GM), or reduced allergenicity (through GM) of a novel food on these attitudes. The second approach will be directed on the development of technological strategies that enables to assess the allergenicity of especially non-GM novel foods. Two scenarios have been chosen: (1) kiwi retrospectively (from-fork-to-farm) and (2) the GM Starlink maize prospectively (from-farm-to-fork). From both products, the history, breeding, production, transport, storage and marketing can be clearly mapped out. The prevalence of kiwi allergenicity is well known. In Starlink maize, the regulatory decisions made to prevent possible allergenic harm are documented extensively. Both approaches together should result in increased knowledge of the public concerns, and of the technical (where, when in the production chain, and what to test) and social possibilities, and chain management implications. Based on this knowledge, measures can be taken to assure the safety of novel foods.

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Digestibility of potential food allergens in TIM-1

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Introduction. Nowadays there is a general tendency to an increased incidence of food allergy. One of the features of potential food allergens is that they are more resistant to gastrointestinal digestion. The digestibility or the stability of food allergens are dependent on different conditions such as the presence of gastric and intestinal secretion products and the presence of a food matrix. Therefore, digestibility or stability of proteins and polypeptides are considered as predictive parameters for *in vivo* allergenicity.

Aim. We investigated whether the TNO dynamic, computer-controlled model of the stomach and small intestine (TIM-1) is a reliable tool to predict the potential allergenicity of (novel) proteins.

Materials and methods. In the present study the fate of known allergens such as ovalbumin, ovotransferrin, lysozyme and peanut proteins (Ara h1-4) in comparison to casein was tested, whether or not in a food matrix, during the passage through the gastric compartment and the small intestine of the TIM-1 system. The gastrointestinal conditions of adults as well as babies were simulated after the intake of a meal. Samples from the experiments were analysed on absorption of nitrogen (as parameter for total digestibility) and on the stability of proteins and polypeptides using gel electrophoresis and immunoblots with serum from sensitised persons.

Results. The preliminary results of the study showed that ovalbumin and lysozyme are very stable during passage through the gastric compartment, irrespective of the presence of a food matrix. In contrast, casein was gradually digested in the stomach to smaller polypeptides. Peanut proteins were broken down rapidly in the stomach if no food matrix was present. However, most peanut proteins were stable in the stomach in the presence of a meal matrix and if the proteins were pre-treated shortly with gastric acid at pH 2. The food allergens were relatively rapidly (further) digested in the small intestine.

Preliminary conclusion. The digestibility or stability of proteins can be investigated under realistic, dynamic, gastrointestinal conditions in TIM-1, including the presence of a complete meal matrix. The resistance of peanut proteins for pepsin after pre-treatment with gastric acid, shows that dynamic conditions (gradual decrease of gastric pH) are essential for the prediction of the protein stability.

Intolerance to dietary biogenic amines: a review

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Background. Biogenic amines are naturally occurring substances in e.g. yeast products, fish, cheese, processed meat, chocolate and alcohol. Since 1967, intolerance reactions to dietary histamine, tyramine and phenylethylamine have been reported.

Objectives. To evaluate the scientific base for the reported intolerance to dietary histamine, tyramine and phenylethylamine.

Methods. In Medline we searched for oral provocation studies in susceptible subjects over the period January 1966 - August 2001. Positive studies had to be double blind, randomised placebo-controlled to be eligible. Subsequently, eligible positive and negative studies were evaluated according to a number of scientific criteria. Studies with severe drawbacks were considered inconclusive.

Results. For the relation between the level of histamine in red wine and adverse reactions after wine consumption, one conclusive negative study was found. Concerning tyramine, two conclusive negative studies were found on the relation between tyramine ingestion and headache attacks in migraine patients. For phenylethylamine, one conclusive negative study on the relation between phenylethylamine in chocolate and the induction of migraine attacks was found. No conclusive positive studies were found at all.

Conclusions. The relation between dietary biogenic amines and food intolerance reactions could not be proved from the scientific literature.

Cross-reactivity of strawberry ns-lipid transfer proteins

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Plant non-specific lipid transfer proteins (LTPs) form a family of 9 kD polypeptides that is widely distributed throughout the plant kingdom. The in vivo function of these proteins is unknown but it has been suggested that they are involved in responses towards stresses such as pathogens, drought, heat, cold and salt. Also, the proteins have been suggested as transporters of monomers for cutin synthesis. LTPs contain 8 conserved cysteines forming 4 disulphide bridges, which makes them highly resistant against high temperatures and pH changes. Recently, LTPs have been identified as allergens in fruits of the *Rosaceae* plant-family (peach, apricot, plum and apple) and in maize. In the Mediterranean area peach LTP is the most frequent cause of food-induced allergic reactions. Due to their extreme resistance to pepsin digestion, LTPs are potential severe food allergens. In addition, cross-reactivity to LTPs from other food-sources may increase the risk of IgE-mediated allergic reactions.

Strawberry fruits (*Fragaria* spp.) belonging to the subfamily *Rosoideae* of the *Rosaceae* family are often mentioned in literature as allergenic. However, only few clinical relevant cases of food-induced allergic reactions to strawberry are known. Using rtPCR, 6 different LTP-isoforms were isolated from fruits of different strawberry cultivars (*Fragaria* spp.). The amino acid identity among the strawberry isoforms ranged from 78%-96%. Surprisingly, the homology in the amino acid sequences with the apple allergen, Mal d 3, was very high, 72%-76%. Homologies with the peach allergen, Pru p 3, and the maize allergen, Zea m 14, were 62%-68% and 54%-59% respectively. A polyclonal rabbit antibody directed against *Arabidopsis* LTP1 detected a 9 kD protein band on Westerns, containing boiled strawberry samples and proteins samples of strawberry isolated by acetone extraction. Cross-reactivity of strawberry extracts and recombinant strawberry ns-LTPs with serum from *Rosaceae* allergic patients will be determined using cap-ELISA. For this, two strawberry isoforms are expressed as rLTPs in *Pichia pastoris*. The identity of cross-reacting proteins will be confirmed by immunoblotting and immunoblot-inhibition experiments.

Other putative allergens which are expressed in strawberry fruit are profilin, peroxidases, pectate lyases and major latex proteins. Also, histamine is mentioned to be present in strawberry fruit. Histamine may cause allergy-like symptoms and therefore histamine concentration in strawberry fruits will also be determined. In summary, the results will be discussed in relation to structural information on the LTP molecules, clinical data, and the effect of the fruit-matrix in which the LTPs are embedded.

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