

ABSTRACTS OF LECTURES AND POSTERS

2ND INTERNATIONAL CONFERENCE

FOOD ALLERGY FORUM

TOWARDS A
FOOD ALLERGY-FREE
WORLD

1-3 APRIL 2019

AMSTERDAM

THE NETHERLANDS



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- abstracts of lectures and posters are grouped separately
- lectures are grouped according to the daily programme
- posters are grouped in an alphabetical order according to the corresponding author

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WELCOME

Food allergy is one of the most common health disorders in the Western world affecting about three per cent of the total population. Food allergy is potentially lethal, and its health impact is higher than that posed by all known chemicals and microbes in food. Additionally, the economic impact of food allergy is immense.

The main objectives of the **FOOD ALLERGY FORUM** are: providing a unique platform to exchange information and experiences on the various aspects of food allergy; reviewing current knowledge related to food allergy; and discussing strategies for prevention and control of food allergy ensuring food safety and protecting human health.

The 2nd international conference of the **FOOD ALLERGY FORUM** focuses on:

- safe food matters: focus on allergens – what is safe
- what makes a protein immunogenic/allergenic
- risk assessment and management of food allergens – what's up
- allergenicity assessment of new or modified food proteins
- food allergy prevention and treatment
- testing and analysis: validation and verification of food allergen control
- consumer analytical devices for food allergens
- the advent of a new era in food allergy control and prevention
- towards a food allergy-free world
- and more...

The 2nd international conference of the **FOOD ALLERGY FORUM** is organised in collaboration with the TNO Immune Health Programme. The conference topics are intended to meet the needs of all stakeholders in the food chain, food researchers, food and healthcare professionals, dietitians, and regulators who want to be updated on the progress and possibilities in this field

High quality speakers, ample time for discussions, and every opportunity to establish rewarding contacts are conference values we want to uphold creating a platform for new initiatives towards a food allergy-free world. You are invited to take part in the discussions with participants from different disciplines and meet business relations in your area. The members of the Advisory Committee wish you an active and fruitful meeting!

On behalf of the Advisory Committee,

Prof. Geert Houben

ADVISORY COMMITTEE

Prof. Geert Houben
conference chair

TNO and University Medical Centre Utrecht, the Netherlands

Dr Joseph Baumert
Prof. Johan Garssen
Prof. Ian Kimber
Astrid Kruijzinga
Dr Gregory Ladics
Dr Stefano Luccioli
Dr Charlotte Bernhard Madsen
Ronald Niemeijer
Dr Bert Popping
Stefan Ronsmans
Dr Paul Turner
Prof. Harry Wichers

University of Nebraska-Lincoln, USA
Nutricia Research and Utrecht University, the Netherlands
The University of Manchester, UK
TNO, the Netherlands
DuPont Industrial Biosciences, USA
U.S. Food and Drug Administration, USA
National Food Institute, Denmark
R-Biopharm AG, Germany
FOCOS GbR, Germany
Coca-Cola Services, Belgium
Imperial College London, UK
Wageningen University & Research, the Netherlands

PROGRAMME AT A GLANCE

MONDAY 1 APRIL 2019

13:00	Opening of the FOOD ALLERGY FORUM 2nd international conference
13:15 – 15:00	Plenary meeting <i>Towards a food allergy-free world</i>
15:00 – 15:30	Networking break & poster viewing
15:30 – 17:20	Session 1 <i>Safe food matters: focus on allergens – what is safe?</i>
17:20 – 17:45	Speed presentations <i>Short presentations by selected poster presenters</i>
17:45 – 18:45	Poster viewing & drinks

TUESDAY 2 APRIL 2019

08:45 – 10:15	Session 2 <i>What makes a protein immunogenic/allergenic?</i>	
10:15 – 10:45	Networking break & poster viewing	
10:45 – 12:30	Session 2 continued	
12:30 – 13:30	Lunchbreak & poster viewing	
13:30 – 15:45	Session 3 <i>Risk assessment and management of food allergens – what's up?</i>	Session 4 <i>Testing and analysis: validation and verification of food allergen control</i>
15:45 – 16:15	Networking break & poster viewing	
16:15 – 18:00	Session 3 continued	Session 5 <i>Consumer analytical devices for food allergens</i>
18:00 – 19:00	Informal get-together & poster viewing	

WEDNESDAY 3 APRIL 2019

08:30 – 11:00	Session 6 <i>Food allergy control and prevention – a quantum leap forward</i>
11:00 – 11:30	Networking break & poster viewing
11:30 – 13:00	Final plenary meeting <i>A food allergy-free world on the horizon</i>
13:00	Closing of the FOOD ALLERGY FORUM 2nd international conference

CONFERENCE PROGRAMME

MONDAY 1 APRIL 2019

13:00 Opening of the **FOOD ALLERGY FORUM** – 2nd international conference
Prof. Geert Houben – conference chair

PLENARY MEETING

TOWARDS A FOOD ALLERGY-FREE WORLD

Fundamental science and technology have reached the stage where sufficient starting points exist for the development and creation of a food allergy-free world.

Chair: Prof. Geert Houben, TNO and University Medical Centre Utrecht, the Netherlands

13:15 *The food allergy landscape: towards a food allergy-free world*

Prof. Geert Houben, TNO and University Medical Centre Utrecht, the Netherlands

13:40 *The socio-economical aspects of food allergens*

Ronald Niemeijer, R-Biopharm AG, Germany

14:00 *Regulatory challenges for allergen-safe foods*

Dr Stefano Luccioli, U.S. Food and Drug Administration, USA

14:20 *Experience gained on allergenicity assessment of (novel) proteins: what should be improved?*

Dr Antonio Fernandez Dumont, European Food Safety Authority, Italy

14:40 *Food allergy treatment and prevention – what's up?*

Dr Jennifer Koplin, Murdoch Children's Research Institute, The Royal Children's Hospital, Australia

15:00 **Networking break & poster viewing**

SESSION 1

See next page.

MONDAY 1 APRIL 2019

SESSION 1

SAFE FOOD MATTERS: FOCUS ON ALLERGENS – WHAT IS SAFE?

How often do we ask ourselves if the food we are eating is safe? Focusing on allergens, what does the concept of safe food include? Different stakeholders will give their view on the question 'what is safe' and define challenges and opportunities.

Chair: Dr Charlotte Bernhard Madsen, National Food Institute, Denmark

15:30 Chair's introduction

15:35 *A changing societal perspective on what is safe enough*
Prof. Ira Helsloot, Nijmegen School of Management, Radboud University Nijmegen, the Netherlands

15:55 *Perceptions of severity: separating fact from fiction*
Dr Paul Turner, Department of Medicine, Imperial College London, UK

16:15 *What is safe: a Canadian regulatory perspective*
Dr Michael Abbott, Bureau of Chemical Safety, Food Directorate, Health Canada, Canada

16:35 *Safely handling allergens: the industry's view on challenges and opportunities*
Stefan Ronsmans, Coca-Cola Services, Belgium / FoodDrinkEurope

16:55 *Unintended presence of allergens in food: how to protect the allergic consumer?*
Dr Sylvia Pfaff, Food Information Service Europe, Germany

17:15 Chair's summary

SPEED PRESENTATIONS

Short presentations (6-minutes) by selected poster presenters to provide an overview of their research and inspire the audience to visit their posters.

17:20 – 17:45

Chair: Astrid Kruizinga, TNO, the Netherlands

P refers to the number of the poster:

- **P1:** *Unprocessed cow's milk suppresses allergic symptoms in a murine model for food allergy – a potential role for epigenetics*
Suzanne Abbring, Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, the Netherlands
- **P3:** *Implementation and optimisation of targeted LC-MS methods for simultaneous detection and quantification of major food allergens*
Dr Antoine H.P. America, Wageningen Plant Research, Wageningen University & Research, the Netherlands
- **P10:** *The feasibility of real-time PCR as a tool for seafood allergen detection and quantification*
Dr Isabel Mafra, REQUIMTE-LAQV, University of Porto, Portugal
- **P13:** *Phylogenetic analysis for discovery of potential new allergens from cassava, mango, papaya and pineapple*
Prof. João Roberto Oliveira do Nascimento, Department of Food Science and Experimental Nutrition, University of São Paulo, Brazil

17:45 – 18:45 **Poster viewing & drinks**

TUESDAY 2 APRIL 2019

SESSION 2

WHAT MAKES A PROTEIN IMMUNOGENIC/ALLERGENIC?

The triggers and immunologic mechanisms behind the break in tolerance and subsequent sensitisation to food allergens are incompletely understood. Further understanding of the mechanisms of tolerance and allergy will help to guide prevention and treatment strategies for food allergy in the future.

Chairs: Prof. Harry Wichers, Wageningen University & Research, the Netherlands
Dr Kitty Verhoeckx, TNO, the Netherlands

08:45 Chair's introduction

08:50 *Allergenicity and immunogenicity: two sides of the same coin?*
Prof. Ronald van Ree, Department of Experimental Immunology, Amsterdam University Medical Center, the Netherlands

09:15 *Impact of food processing on immunogenicity: time to define food processing-associated molecular patterns (FAMPs)?*
Prof. Harry Wichers, Wageningen Food & Biobased Research, Wageningen University & Research, the Netherlands

09:35 *Electric fields processing: novel perspectives on food allergenicity*
Dr Ricardo Pereira, Centre of Biological Engineering, University of Minho, Portugal

09:55 *The role of protein processing and digestion in food allergy*
Dr Eva Untersmayr-Elsenhuber, Institute of Pathophysiology and Allergy Research, Medical University of Vienna, Austria

10:15 **Networking break & poster viewing**

Special focus on assessing the allergenicity of new or modified food proteins

10:45 *Improving allergy risk assessment strategy for new food proteins (ImpARAS) project: introduction and achievements*
Dr Kitty Verhoeckx, TNO, the Netherlands

11:05 *Influence of physicochemical parameters on the allergenicity of protein families*
Dr Joana Costa, REQUIMTE-LAQV/Faculty of Pharmacy, University of Porto, Portugal

11:25 *Adverse outcome pathway (AOP)-based in vitro approaches for the evaluation and prediction of the sensitising potential of food proteins*
Dr Daniel Lozano-Ojalvo, Department of Pediatrics, Icahn School of Medicine at Mount Sinai, USA

11:45 *Can you predict a novel allergen in an animal model?*
Dr Katrine Lindholm Bøgh, National Food Institute, Technical University of Denmark, Denmark

12:05 *Defining the targets for the assessment of IgE-mediated allergenicity of new or modified food proteins*
Dr Ben Remington, TNO, the Netherlands

12:25 Chair's summary

12:30 **Lunch break & poster viewing**

TUESDAY 2 APRIL 2019

SESSION 3

RISK ASSESSMENT AND MANAGEMENT OF FOOD ALLERGENS – WHAT'S UP?

By identifying risk factors during processing as well as determining appropriate 'safe' thresholds of allergenic proteins, the food industry can take increasingly proactive steps to avoid direct or cross-contamination as well as ensuring that their products are appropriately labelled and identified for those at risk. This session covers a range of critical topics in this area identifying both knowledge and data gaps.

Chairs: Stefan Ronsmans, Coca-Cola Services, Belgium
Dr Gregory Ladics, DuPont Industrial Biosciences, USA

13:30 Chair's introduction

13:35 *No two the same: comparative overview on allergen labelling regulations*
Cesare Varallo, Food Lawyer and Founder of Foodlawlatest.com, Italy

14:00 *Analytical detection methods versus VITAL reference values: what are the challenges and solutions?*
Dr Joseph Baumert, Food Allergy Research and Resource Program, University of Nebraska-Lincoln, USA

14:20 *Thresholds and cofactors in allergic reactions: lessons from the TRACE study*
Dr Shelley Dua, Department of Medicine, University of Cambridge, UK

14:40 *Accepting and comparing risks – risk assessment and risk management decision cases in Sweden: a case study*
Dr Ylva Sjögren Bolin, National Food Agency, Sweden

15:00 *A quantitative risk-based approach to food allergen management*
Dr Stella Cochrane, Safety & Environmental Assurance Centre, Unilever, UK

15:20 *Assessing the efficacy of cleaning programmes to prevent allergen cross-contact*
Dr Lauren Jackson, U.S. Food and Drug Administration, USA

15:45 **Networking break & poster viewing**

16:15 *A retailer's view on food allergen management*
Ika Van de Pas, Albert Heijn, the Netherlands

16:35 *Thresholds, food intake, severity, particulates, sampling and analytical uncertainties: can we account for all aspects in risk assessment 2.0?*
Dr Charlotte Bernard Madsen, National Food Institute, Technical University of Denmark, Denmark

Special focus on proactive intervention

17:00 *Early introduction of foods to prevent food allergy*
Dr Ted Klok, Isala Hospital Deventer, the Netherlands

17:20 *Microbiome modulation as a tool to manage allergic disorders*
Prof. Johan Garssen, Nutricia Research and Utrecht University, the Netherlands

17:40 *Prevention of food allergic reactions – the clinical perspective*
Dr Thuy-My Le, Department of Dermatology and Allergology, University Medical Center Utrecht, the Netherlands

18:00 Chair's summary

18:00 – 19:00 **Informal get-together & poster viewing**

TUESDAY 2 APRIL 2019

SESSION 4

HOW THE USE OF NEW ANALYTICAL TOOLS BY COMPETENT AUTHORITIES WILL IMPACT ALLERGEN RISK MANAGEMENT

Testing for food allergens is an expensive endeavour. Many companies that take allergen management seriously, spend a significant amount of their quality control budget on analysis for the presence of food allergens. And yet, typically only a few food allergens are tested for. Competent authorities have now moved towards a new technology which will look at a wide range of food allergens in a single test. Several groups are currently working to develop and validate such methods.

Chair: Dr Bert Popping, FOCOS GbR, Germany

13:30 Chair's introduction

13:35 *Establishing measurement systems for achieving comparable data in risk assessment relevant units for the precautionary labelling of food*
Prof. Hendrik Emons, European Commission, Joint Research Centre, Belgium

13:55 *Food safety and food authenticity by peptide mass spectrometry – Constitution of a new § 64 LFGB working group for method validation and standardisation*
Dr René Becker, Federal Office of Consumer Protection and Food Safety (BVL), Germany

14:15 *Evaluation of performance criteria for allergen measurement by ELISA*
Dr Markus Lacorn, R-Biopharm AG, Germany

14:35 *Quantitative assessment and testing, an industry perspective*
Dr Neil Buck, General Mills, Switzerland

14:55 *Review of suitability of analytical methods for measuring action levels determined by various guidelines*
Dr Thomas Holzhauser, Division of Allergology, Paul Ehrlich Institute, Germany

15:15 *Overview over food allergen methods in respect to allergen risk management and certification schemes*
Dr Bert Popping, FOCOS GbR, Germany

15:40 Chair's summary

15:45 **Networking break & poster viewing**

SESSION 5

See next page.

TUESDAY 2 APRIL 2019

SESSION 5

CONSUMER ANALYTICAL DEVICES FOR FOOD ALLERGENS

A new kind of testing devices has emerged, which place otherwise complicated laboratory testing into the hands of consumers. Devices are based on different analytical principles and include immunological tests, molecular imprinting and DNA-/RNA-based capturing methods. However, to make such devices suitable for untrained consumers, the procedures to analyse and read results have to be simple and fail-safe.

Chair: Dr Bert Popping, FOCOS GbR, Germany

16:15 Chair's introduction

16:20 *Overview of consumer analytical devices for gluten and allergen detection*
Gina Ross, Wageningen Food Safety Research, the Netherlands

16:40 *The Nima sensor: a consumer device for the detection of gluten or peanut allergens in food*
Francisco Dias Lourenco, Nima, USA

17:00 *Evaluation of the Nima sensor for detection of gluten residue in incurred food matrices*
Dr Joseph Baumert, Food Allergy Research and Resource Program, University of Nebraska-Lincoln, USA

17:20 *Interactive panel discussion*

Panelists: Dr Bert Popping, FOCOS GbR, Germany (moderator)
Gina Ross, Wageningen Food Safety Research, the Netherlands
Francisco Dias Lourenco, Nima, USA
Dr Joseph Baumert, University of Nebraska-Lincoln, USA
Sabine Schnadt, Deutscher Allergie- und Asthmabund, Germany

18:00 – 19:00 **Informal get-together & poster viewing**

WEDNESDAY 3 APRIL 2019

SESSION 6

FOOD ALLERGY CONTROL AND PREVENTION – A QUANTUM LEAP FORWARD

With the advent of new knowledge and technologies, a new era is commencing. What are the challenges and opportunities for treatment and prevention of food allergy?

Chair: Prof. Harry Wichers, Wageningen University & Research, the Netherlands

08:30 Chair's introduction

08:35 *The impact of immune interventions: a systems biology strategy for predicting adverse and beneficial immune effects*

Dr Jolanda van Bilsen, TNO, the Netherlands

08:55 *Developing a genetic risk index for peanut allergies*

Dr Denise Daley, Centre for Heart Lung Innovation, St. Paul's Hospital, Canada

09:15 *Epigenetic modifications – promising tools in the management of allergic diseases*

Dr Jörg Tost, Laboratory for Epigenetics and Environment, Centre National de Recherche en Génomique Humaine, France

09:35 *The first reference genome sequence for bread wheat and its implications for wheat allergy and intolerance*

Dr Manuel Spannagl, Plant Genome and Systems Biology, German Research Center for Environmental Health, Germany

09:55 *Paths to engineering peanut for reduced allergenicity*

Prof. Peggy Ozias-Akin, Department of Horticulture, University of Georgia, USA

10:15 *Food allergy prevention and treatment by targeted nutrition*

Dr Ralf Heine, Nestlé, Switzerland

10:35 *A real-world use of digital ledgers for controlling consumer allergen risk: a case study*

James Flynn, Primority Ltd., UK

10:55 Chair's summary

11:00 **Networking break & poster viewing**

FINAL PLENARY MEETING

See next page.

WEDNESDAY 3 APRIL 2019

**FINAL PLENARY MEETING
A FOOD ALLERGY-FREE WORLD ON THE HORIZON**

What does the (near) future hold?

Chair: Prof. Geert Houben, TNO and University Medical Centre Utrecht, the Netherlands

11:30 Chair's introduction

11:35 *Where are we in 2025 in protecting existing food allergy sufferers?*
Prof. Samuel Godefroy, Food Science Department, Laval University, Canada

11:55 *Where are we in 2025 in curing food allergy?*
Prof. André Knulst, Department of Dermatology and Allergology, University Medical Center Utrecht, the Netherlands

12:15 *Where are we in 2025 in preventing food allergy?*
Dr Marta Krawiec, Department of Respiratory Medicine and Allergy, King's College London, UK

12:35 Q&A

12:45 *TripAdvisor: Top stopovers on our journey towards a food allergy-free world learned at the*
FOOD ALLERGY FORUM
Prof. Geert Houben, TNO and University Medical Centre Utrecht, the Netherlands

13:00 Closing of the **FOOD ALLERGY FORUM** – 2nd international conference

Take your packed lunch to eat along the way!

LECTURES

MONDAY 1 APRIL 2019

**PLENARY MEETING
TOWARDS A FOOD ALLERGY-FREE WORLD**

Fundamental science and technology have reached the stage where sufficient starting points exist for the development and creation of a food allergy-free world.

THE FOOD ALLERGY LANDSCAPE: TOWARDS A FOOD ALLERGY-FREE WORLD

Geert Houben

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Food allergy is among the most prevalent disorders in the Western world. It is a cause of loss of health that outreaches that of known chemicals and microbes in food, particularly because of its enormous impact on the quality of life of food allergy sufferers and their environment. The loss of health due to food allergy is estimated to be higher than that of for instance skin cancer, Parkinson's disease or cardiac arrest or at a same order of magnitude as prostate cancer, asthma or rheumatoid arthritis. In a recent prospective study in the Netherlands, 4 emergency hospital visits per 100 food allergic individuals per year were recorded. For comparison: less than 100 hospitalisations due to food infections are recorded per year for the whole population of over 17 million of people in the Netherlands.

Food companies invest strongly in preventive measures and risk management, yet several studies demonstrate that despite these investments, up to 30 to almost 50% of all food recalls are allergen related. Although it will be challenging to achieve, we need to reduce the burden of food allergy to a minimum and have to have the ambition to strive for a food allergy-free world. There are various trails we can follow to reach our destination, but it likely is advisable to travel via the following 3 waypoints:

- *Protection of existing food allergic consumers.* The main focus of food allergy management is the avoidance of the intake by the food allergy sufferer of the substance that he or she is allergic to. This requires a faultless identification and diagnoses of the allergic patient and measures to provide them with the possibility and ability to identify unsafe foods and have sufficient choice in safe food products (allergy management). It also requires companies in the food chain to be able to adequately assess and manage allergen risks and make adequate risk communication decisions (allergen management).
- *Prevention of the introduction of new strong allergenic foods.* The growing world population and the increasing impact of man on the environment demands for changes in agricultural practices and food supply. Development and use of new or improved (climate-resistant) crops, currently unused by-products and more sustainable alternative food protein sources can significantly contribute to improving the sustainability of our food supply. However, allergenicity is a major potential health risk for products based on or containing new or modified proteins and adequate and accepted methods and standards for establishing the allergenicity of new or modified proteins are required to ensure safety and avoid the introduction of new strong allergenic foods.
- *Cure and prevention of food allergy.* Curing food allergies or preferably even protecting susceptible (particularly young or even unborn) individuals against the development of food allergies would of course provide the ultimate solutions. This will require interventions in immune functions and balances. However, a well-balanced immune system is key for overall health and well-being. Methods and tools for careful assessment of risks and benefits and accurate monitoring of immune health interventions are currently lacking to a major extend and are needed to develop and apply safe approaches to cure or prevent food allergies.

During the past decades, an enormous progress has been made in understanding mechanisms in food allergy and tolerance, the effects and risks of allergenic foods and proteins on food allergy sufferers and those susceptible to the development of food allergy, and in allergen and allergy management approaches. Today, fundamental science and technology have reached the stage at which sufficient starting points exist to develop the ultimate solutions needed. Multidisciplinary collaboration and action between industry, universities, clinical centres, patient organisations and authorities, can take away remaining hurdles and make us take the steps needed to keep us firmly on the road mapped *TOWARDS A FOOD ALLERGY FREE WORLD*.

SOCIO-ECONOMICAL ASPECTS OF FOOD ALLERGENS

Ronald Niemeijer

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About 2-5% of the adult population suffers from one or more food allergies. Amongst children these numbers seem to be twice as high. Although not a food allergy, celiac disease is often mentioned in the same context. It is estimated around 1% of the population suffers from this gluten triggered auto-immune disease. Extrapolating this number to the number of households, this would mean that the number of people affected by a food allergy (directly or indirectly) is probably 3-4 times higher. On top of that, if people are asked if they think they have a food allergy up to 25-30% of them answer positively.

Food allergies have a significant socio-economic impact. Individuals suffering from a food allergy and their family or household members are facing several additional costs. In the case of emergencies and hospitalisation, additional healthcare expenditures are made. But beyond those direct costs, individuals with a food allergy face various indirect costs, such as loss of productivity and quality of life. To protect allergic consumers and to assure consumers are informed in a correct way about the presence of allergens in their food, labelling legislation is in place in a growing number of countries all around the world. This legislation reflects to some extent the local differences in occurrence of allergens but are almost all based on the so called 'Big-8' of food allergens (milk, egg, fish, crustacean, tree nuts, peanuts, wheat, and soybeans). For gluten (or 'gluten-free'), separate legislation is in place in many countries. To meet the legislation and customer requirements, food producers should have a solid allergen management in place. This means of course the food industry has to invest in quality assurance with respect to food allergens, e.g., testing, but also in production capacities (dedicated production lines or at least an effective cleaning regime). Also sourcing of raw materials and monitoring of suppliers may lead to higher production costs. On the other hand, the increasing number of consumers interested to buy 'allergen-free' (or gluten-free) products is increasing rapidly. In fact, this is one the growth drivers in the food industry and offers new possibilities for the food industry.

This presentation will give an overview of the socio-economical aspects of food allergens from the consumers' and the industry's point of view. Current and potential future tools in allergen management and food allergen testing, and their pros and cons are discussed.

REGULATORY CHALLENGES FOR ALLERGEN-SAFE FOODS

Stefano Luccioli

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Food allergies are increasing in prevalence and pose a significant public health burden. They are not only a leading cause of life-threatening anaphylaxis in the community but are also associated with a substantially reduced quality of life for food allergic individuals and their families. At present, there are no proven treatment options for food allergies; thus, the only effective management option to avoid harm is strict avoidance of all foods that contain the allergen. In this regard, food safety regulatory agencies have an important mission to protect the food allergic public by ensuring that allergens of public health importance are properly identified in labelled food products and that unsafe products with undeclared allergen hazards are removed from the food supply. Yet, guaranteeing 'allergen-safe' foods that are always properly labelled and not hazardous to the allergic population is not straightforward.

Allergens are commonly present in foods both intentionally and unintentionally and at highly variable levels. Despite established allergen labelling laws, food products with undeclared or misleading statements about allergens have become a leading cause of food recalls and may also be contributing to an unappreciated number of allergic reactions in the consumer population. Moreover, what levels of allergen are hazardous vs. safe, i.e., allergen thresholds, have not been universally defined. Given the potential for severe reaction from any one allergen exposure and the very wide sensitivity range in responders to allergen dose exposures within the population, it is difficult to establish practically enforceable allergen threshold doses or action levels that are inconsequential to health and would not elicit risk for potentially severe reactions in highly sensitive members of the allergic population. Agreement on some acceptable risk level is therefore necessary but this has been hindered in large part by a general lack of understanding of allergen risks or thresholds by the public and beliefs that no risk of allergic reaction is acceptable. An issue that has come into the forefront is the fact that allergens unintentionally incorporated into product by way of cross-contact are not subject to most labelling regulations. The levels of allergen incorporated into foods due to cross-contact can vary considerably depending on the physical properties of the food and allergen, the equipment used to process the food, and effectiveness of allergen control strategies.

A lack of consensus on threshold levels has led to proliferation of advisory or precautionary allergen labelling statements that may not accurately indicate the risk of allergen hazard in the product to consumers. Research based on quantitative risk assessment approaches is emerging to better understand and model data on threshold responses that are consequential to the health of allergic populations. These data are beginning to influence the regulatory risk assessment landscape and impact decisions on what allergen-containing products may be allergen-safe or not.

EXPERIENCE GAINED ON ALLERGENICITY ASSESSMENT OF (NOVEL) PROTEINS: WHAT SHOULD BE IMPROVED?

Antonio Fernandez Dumont

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The allergenicity assessment of proteins is paramount in food safety. Because there is not a single method that on its own enables to predict the allergenic potential of a protein, a cumulative body of evidence, in a so-called weight-of-evidence approach, is in place to reduce uncertainty and improve the reliability of predictions. International guidelines defined by Codex Alimentarius and embedded into EFSA guidance documents provide a detailed description of the principles to follow when performing such assessments. The evidence of different nature necessary for the safety of a food protein is as follows: knowledge on the source of the protein and on the protein itself, bioinformatics analysis, in vitro protein degradation studies and, on a case-by-case basis, specific serum screening and cell-based assays or animal models.

Food safety assessments should rely on evidence at the cutting edge of science, which requires continuous updates of guidelines requirements. EFSA monitors new scientific developments in the area and is committed to incorporating new scientific developments in its risk assessment process, when appropriate. For example, the first comprehensive scientific evidence-based strategy for the safety assessment of proteins to cause coeliac disease has been recently developed by EFSA. Looking at the future, there are different aspects of the current safety assessment strategy requiring additional research and discussion before their potential inclusion in the overall risk assessment process. These aspects include the future development of more robust validated bioinformatic approaches, more refined in vitro protein digestion protocols, more predictive cell-based approaches. In addition, HLA-phenotyping in humans will provide insights for the development of more advanced and effective risk assessment tools based on state-of-the-art knowledge. All these aspects will be further described in detailed during the presentation.

FOOD ALLERGY TREATMENT AND PREVENTION – WHAT’S UP?

Jennifer Koplin

Murdoch Children’s Research Institute, The Royal Children’s Hospital, Australia
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IgE-mediated food allergy, a potentially life-threatening condition, currently affects up to 10% of infants and 5% of older children. A rising rate of hospital admissions for food allergy-related anaphylaxis has been noted in multiple countries around the world. Identification and implementation of effective food allergy prevention and treatment strategies is thus imperative.

The past 10 years has seen substantial progress in understanding risk factors for food allergy. Large population-based observational studies revealed several factors associated with an increased risk of food allergy, including delayed introduction of allergenic foods into the infant diet, an impaired skin barrier in infancy, lower infant vitamin D levels, and reduced or altered microbial exposure in infancy. Several of these factors are now being tested in rigorous randomised controlled trials. The results from the first of these trials are already available. A recent landmark study showed an ~80% reduction in peanut allergy prevalence in a select group of high-risk children (i.e., those with early signs of allergy – infantile eczema or egg allergy) with early and regular consumption of peanut starting in the first year of life. As a result, expert guidelines around the world now recommend that infants, particularly those at high risk, begin consuming peanut products from as early as 4-6 months of age. The past decade has also seen a shift away from food allergen avoidance as the mainstay of treatment for food allergy, towards attempts to actively induce tolerance in food allergic individuals. Several different routes of immunotherapy have been investigated in clinical trials and have shown varying degrees of success in achieving desensitisation, defined as an increased threshold for reaction to the food allergen. These include oral immunotherapy (OIT), with or without adjuvant therapy, sublingual immunotherapy, and, more recently, epicutaneous immunotherapy.

This presentation will examine the current evidence and state-of-play for these food allergy prevention and treatment strategies with regards to research and implementation of each of these strategies. The discussion of food allergy prevention will focus on five key risk factors which have been or are currently being tested in randomised controlled trials: (i) timely introduction of allergenic foods into the infant diet; (ii) maternal consumption of allergenic foods during pregnancy and breastfeeding; (iii) infant skin barrier and the role of moisturisers in early life; (iv) infant vitamin D levels and the role of vitamin D supplementation; and (v) microbial exposure in early life.

MONDAY 1 APRIL 2019

**SESSION 1
SAFE FOOD MATTERS: FOCUS ON ALLERGENS – WHAT IS SAFE?**

How often do we ask ourselves if the food we are eating is safe? Focusing on allergens, What does the concept of safe food include? Different stakeholders will give their view on the question 'what is safe' and define challenges and opportunities.

A CHANGING SOCIETAL PERSPECTIVE ON WHAT IS SAFE ENOUGH

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For thousands of years accidents were simply an act of some unknown god that was probably angered by your own fault. Later on, accidents were your own fault. Both perspectives are referred to as the blame culture. It is only recently that we consider risk as a combination of chance and effect. Accidents therefore can largely be prevented by taking sensible measures to tackle the biggest risk. Some risks, and thus accidents, are acceptable by this approach. This we call the risk culture.

The last 15 years or so, a new perspective on risk has emerged in the domain of policy making in the rich Western world. All accidents are considered proof of the failure of a safety system instead of an occasional expression of 'bad luck'. So, for example allergy is no longer an individual problem that calls for extreme caution of people suffering from it but a potential failure of food producers that do not prevent contamination. A new blame culture has arisen. This approach has benefits for those exposed to exotic risks, but the costs may prevent taking sensible measures that benefit a larger portion of society. The tension between the risk culture and the new blame culture warrants a better look at risk regulation reflex.

PERCEPTIONS OF SEVERITY: SEPARATING FACT FROM FICTION

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Food allergy is the commonest cause of life-threatening allergic reactions, affecting up to 6% of the population. Although anaphylaxis has been defined as a 'severe, life-threatening generalised or systemic hypersensitivity reaction', data indicate that the vast majority of food-triggered anaphylaxis reactions are not life-threatening. Nonetheless, severe life-threatening reactions do occur and are unpredictable. This contributes to social restrictions and anxiety which impact significantly on allergic sufferers and their families.

The biggest challenge in managing individuals with food allergy is our current inability to reliably identify those people who are at risk of severe, life-threatening reaction. Our knowledge regarding the mechanisms of food allergic reactions in humans is limited, partly due to the fact that animal models are of very limited relevance. We cannot explain why one person will develop life-threatening anaphylaxis to under 1/70 of a peanut, while another will develop only localised itch after eating 3-4 peanuts. Allergy tests (skin prick test size, specific IgE antibody to either a total allergen or allergen component) do not predict the severity or the sensitivity of an individual. For example, a patient with small skin test can have life-threatening allergic reaction, while a patient with a big skin test may have a mild reaction limited to the skin.

In this presentation, we will review the evidence regarding factors that might be used to identify those at most risk of severe allergic reactions to food, and the consequences of misinformation in this regard. For example, a significant proportion of food-allergic children also have asthma, yet almost none will experience a fatal food-allergic reaction; asthma is not, in itself, a strong predictor for fatal anaphylaxis. The relationship between dose of allergen exposure and symptom severity is unclear. While dose appears to be a risk factor in at least a subgroup of patients, studies report that individuals with prior anaphylaxis do not have a lower eliciting dose than those reporting previous mild reactions. It is therefore important to consider severity and sensitivity as separate factors, as a highly sensitive individual will not necessarily experience severe symptoms during an allergic reaction.

WHAT IS SAFE: A CANADIAN REGULATORY PERSPECTIVE

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This presentation will provide an overview of the approach taken by Health Canada for health risk assessments involving undeclared or improperly declared food allergens in pre-packaged foods. The health risk assessment process takes into account exposure (amount of exposure and likelihood of exposure) as well as hazard (whether the food could provoke an adverse reaction if consumed). Relevant factors considered include how the food is labelled, how much of the food was produced and distributed, shelf life, which allergen(s) are present and in what type of food, etc. An important element of the health risk assessment is the amount of allergen present in a serving of the food; the amount a consumer would be exposed to if they were to consume it. This amount can be compared to data such as LOAELs/NOAELs or ED values to determine the level of hazard present.

The presentation will also include discussion of some limitations of using LOAELs/NOAELs and ED values in health risk assessment and the ongoing challenge for regulators who must determine whether a food under investigation is safe and if not, how large a risk the food represents.

SAFELY HANDLING ALLERGENS, THE INDUSTRY'S VIEW ON CHALLENGES AND OPPORTUNITIES

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Food industry is on an ongoing basis being confronted with food safety challenges of all kinds. For these challenges, well established management systems have been used throughout history, consisting of well-defined and acknowledged management system elements, prerequisite programs and above all the powerful application of the hazard analysis and critical control points (HACCP) principles. This approach not only guarantees the food safety integrity of the foods produced daily. Striving for compliance with these principles also triggers and maintains a process of continuous learning and improvement, which in the end benefits the final consumers of the produced foods.

While food safety risks, such as the presence of foreign materials, the impact of microbiology and the challenge of food contaminants, typically have a generally accepted limit or benchmark, most often specified in the applicable food legislation, this is not the case for food allergens. The legislative framework on allergens is mostly only qualitative in kind, without generally agreed upon limits. Considering also the ever-increasing complexity of the global supply chains of ingredients and finished products, this absence of commonly agreed upon quantitative risk management limits poses a particular challenge to the food industry when it comes to allergens. It also hampers the application of the generally applied HACCP approach, and the benefits and learnings that come from its application.

As this session of the Food Allergy Forum focusses on the question 'what is safe' in the context of food allergies, the presentation also contrasts and compares approaches that have been applied in other areas of food safety risk assessment – and management where, comparable to the field of food allergies, an absolute zero residual risk may equally not be achievable.

UNINTENDED PRESENCE OF ALLERGENS IN FOOD: HOW TO PROTECT THE ALLERGIC CONSUMER?

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Food allergies can range from merely irritating to life-threatening. In Europe, the Anaphylaxis Campaign of UK estimates 10 deaths per year due to allergens. Although these reactions are caused mainly by unknown (e.g., 'forgotten') ingredients that are present in high amounts in the foods, also unintentional allergen presence has to be considered by the food industry. The presentation will give an outline why an effective allergen management system and the risk assessment is important, on the background of risk assessment, and how risk assessment is carried out with some examples of recalled foods:

- The total amount of consumed protein is important for an adverse reaction (= medical threshold). It differs for different individuals and in certain situations (e.g., stress, illness, alcohol consumption, sport activities, etc).
- The medical threshold of tested allergic consumers is transferred to a reference dose which shall protect 99% (respectively 95%) of the allergic population.
- Scientific knowledge states that if a reaction occurs at this value it will be mild.
- Risk assessment operates with this reference dose.
- The dose is transferred into action levels according to the portion size.

The audience will get an insight into the allergen management, risk assessment and labelling of unintentional allergen presence.

TUESDAY 2 APRIL 2019

**SESSION 2
WHAT MAKES A PROTEIN IMMUNOGENIC/ALLERGENIC?**

The triggers and immunologic mechanisms behind the break in tolerance and subsequent sensitisation to food allergens are incompletely understood. Further understanding of the mechanisms of tolerance and allergy will help guide prevention and treatment strategies for food allergy in the future.

ALLERGENICITY AND IMMUNOGENICITY: TWO SIDES OF THE SAME COIN?

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Unfortunately, the abstract was not received in time to be published in the book of abstracts.

IMPACT OF FOOD PROCESSING ON IMMUNOGENICITY: TIME TO DEFINE FOOD PROCESSING-ASSOCIATED MOLECULAR PATTERNS (FAMPS)?

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The increased prevalence of allergies is explained as a very multi-factorial problem, for which changes in our lifestyle and dietary patterns often are held responsible. Epidemiologically, correlations are being made to increased hygiene, vaccination campaigns, increased usage of antacids and antibiotics, rural vs. urban living environment, switch to a 'Western' diet, decreased diversity of intestinal microbiota, intestinal dysbiosis, to mention a few, without the pretention of being exhaustive.

In the initiation and/or 'education' of the immune system, archetypical microbial structures are involved that are classified as 'PAMPs' (in the 'initiation' connotation) or 'MAMPs' (in the 'educational' connotation). In addition, the concept of 'DAMPs' was coined to indicate endogenously formed activators of immune responses, e.g., upon trauma. Modified protein structure is an important factor in DAMP-signalling, which is involved in a number of pathologies.

(Food) allergic reactions occur for proteins that are contained in such foods. Nearly all foods that we consume, have been processed (often via applying heat), either industrially or in a home-setting, which will lead to structural changes in such proteins, e.g., the formation of Maillard reaction products, and unfolding and aggregation. In the preparation of proteins for pharmaceutical purposes, care is taken to minimise, e.g., shear-related damage to their structure, to limit immunogenicity. Such structure changes may be regarded as 'damage', when the native protein is taken as the starting point. There are strong indications, based on *in vivo* experimentation, that processing of proteins under conditions that are similar to typical food processing, does have an effect on their structural properties as well as on their antigenicity, including their propensity to initiate allergic responses. The processing-related structural alterations in proteins will be discussed with allergic responses to peanut or milk proteins as examples and leads to the postulation of the Food-processing Associated Molecular Pattern (FAMP-)concept.

ELECTRIC FIELDS PROCESSING: NOVEL PERSPECTIVES ON FOOD ALLERGENICITY

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Ohmic heating (OH) is establishing a solid foothold in the innovative food processing due to its fast, direct and volumetric heating with remarkable implications in food quality, safety (i.e. non-thermal microbial inactivation) and energy efficiency. Over the last decade novel insights have been brought about the interactions of electric variables – such as electric field intensity and electrical frequency – on dynamic behaviour of important food proteins during their thermal denaturation process. Protein rich-fractions of beta-lactoglobulin (b-LG) has been used as model to evaluate conformational transitions, distribution of secondary structures and aggregation patterns imposed by different electric field processing protocols. It is now an evidence that combined effects of electrical and thermal treatments brought by OH can reduce thermal denaturation and aggregation of b-LG but also interfere with their unfolding transitions and protein-protein interaction properties, giving rise to aggregates with different morphologies and distinct physical and chemical internal networks. Knowing that a great majority of important food proteins are elicitors of allergic reactions, is then essential to understand the implications that the non-thermal effects of electric fields have on the potential allergenicity of the food proteins. There is a lack of research regarding the impact of OH on immunoreactivity of proteins, and the outcomes of traditional thermal processing should not be automatically assumed – due to internal heating (i.e., different levels of thermal load) and effects of electric fields on biophysical state of proteins. Our findings unravel that OH brings a new paradigm for the thermal denaturation of protein-rich fractions with potential to change resilience of protein aggregated structures to gastrointestinal digestion, which can bring potential consequences on allergic response during intestinal absorption that need to be thoroughly assessed.

Acknowledgments

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THE ROLE OF PROTEIN PROCESSING AND DIGESTION IN FOOD ALLERGY

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To ensure consumers' safety, it is essential to define characteristics associated with allergenicity of food proteins. Before interaction of potential allergens with immune induction sites in the gastrointestinal tract, proteins are exposed to, e.g., posttranslational modification or food matrix interactions. Moreover, also food processing and digestion alter protein characteristics. Thus, also enzymatic and thermal stability might differ between dietary proteins and contribute to allergenicity. Trying to mimic the physiological protein degradation, gastrointestinal digestion assays are performed at low pH levels with gastric enzymes followed by incubation with pancreatic proteases at neutral pH levels. However, when considering the situation in patients, a large variety of different factors influence the outcome of these digestion assays. Moreover, also food processing and post-translational protein modifications might change protein structure and digestibility, influencing the potential to elicit a type 2 immune response.

Representing the first site of protein digestion especially gastric digestion was found to contribute essentially to protection against food allergy development. Either under conditions of gastric hypoacidity or after surgical elimination of the gastric digestion an enhanced sensitisation potential of food allergens as well as an aggravation of pre-existing symptoms were reported. Of interest, facilitated gastric protein digestion due to specific posttranslational protein modification by protein nitration was found to be associated with reduced sensitisation capacity and food allergy prevention in experimental models.

Profound knowledge of these mechanisms might not only enable enhanced consumers' and patients' safety but may additionally lead to innovative novel treatment strategies of type 2 mediated diseases.

Acknowledgements

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IMPROVING ALLERGY RISK ASSESSMENT STRATEGY FOR NEW FOOD PROTEINS (IMPARAS) PROJECT: INTRODUCTION AND ACHIEVEMENTS

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As the world population grows and the impact of food supply practices on the environment is immense, the demand for new sustainable proteins will increase. However, proteins are also associated with allergies. Consequently, any new protein product requires evaluation of the risk of allergenicity that it poses before it can be used for human consumption. With approx. 17 million food allergy sufferers, and more potentially at risk of developing allergies in Europe alone, the scale of the issue cannot be underestimated. Unfortunately, there are currently no validated methods that reliably and consistently predict the allergenicity of a protein. ImpARAS, which stands for Improving Allergenicity Risk Assessment, is a COST Action that was running from 2014-2018. ImpARAS aimed to improve the assessment of protein allergenicity and served the needs of all those involved in the process of developing, assessing and using novel proteins. ImpARAS is a network of more than 300 members coming from industry, universities, research institutes, hospitals and the European Food Safety Authority from 30 countries.

ImpARAS had 4 working groups (WG). WG1 identified the gaps in knowledge of the physical/chemical properties of proteins impacting their allergenicity. Additionally, WG1 studied in more detail selected protein families, comparing strong and weakly allergenic proteins and physiological differences between known allergenic protein families. WG2 reviewed existing knowledge concerning protein uptake and bioavailability, allergen exposure and the activation of the innate and adaptive immune mechanisms and processes. The available information was structured according to the Adverse Outcome Pathways (AOP) concept and *in vitro* methods for studying these were linked to it. WG3 reviewed animal models and how we can correlate *in vivo* with *in vitro* findings. WG4 identified the gaps in the current risk assessment strategy and defined possible risk management targets for the assessment of IgE mediated allergenicity of proteins and identified their implications on future methods development.

The impact of ImpARAS is: a European network of leading institutes on food allergy; input on allergenicity assessment strategies to EFSA, via the EFSA focus group on allergenicity; training and education of young European scientists; an AOP for food allergy sensitisation; awareness of the importance of allergenicity assessment of novel foods amongst many stakeholders; and participation in EU commission meeting on future needs in food safety research.

INFLUENCE OF PHYSICOCHEMICAL PARAMETERS ON THE ALLERGENICITY OF PROTEIN FAMILIES

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Most allergens are known to belong to a restricted number of protein families sharing a certain pattern of biochemical functions among them. This fact seems to support the idea that allergens possess special features and not every protein can become allergenic [1]. So far, there are several studies reporting the effect of different physicochemical properties on distinct allergens, but most of them are made at individual basis. With this work, it is intended to investigate published evidences to which extent different physicochemical features might affect the allergenicity of proteins from different families of food allergens. In the case of plants, families containing the most relevant allergens were extensively analysed, namely the 2S albumins, nonspecific lipid transfer proteins (nsLTP), cereal alpha-amylase trypsin inhibitor (ATI) and cereal prolamin families (members of the prolamin superfamily), legumins and vicilins (members of the cupin superfamily), profilins and pathogenesis related (PR)-10 proteins. Members of each protein family were fully investigated considering a specific set of physicochemical parameters that included: post-translational modifications (glycosylation, phosphorylation, hydroxylation and deamidation); protein structure and organisational level; stability to heat, light/radiation pressure, mechanical and chemical activities; glycation; aggregation; lipid binding; lipid interactions; and resistance to digestion.

The extensive analysis of the available literature provided several evidences about the impact of such features, highlighting that within each protein family of plant foods, the allergenic potential of its members seems to follow the same tendency, although occasional exceptions could be observed. At individual basis, some parameters like heat and structural stabilities, as well as, resistance to proteolytic activity, are considered of crucial relevance for protein allergenicity. However, the influence of food processing and the matrix effect are still far from being totally understood. Presently, most of the methods used for allergenicity assessment are applied indirectly, via serum/plasma IgE-binding rather than directly via oral provocation, supporting that there is a significant gap between the influence of each physicochemical parameter and their real clinical impact. New tools for allergenicity assessment are currently being proposed (e.g. animal models), but still there is a certain degree of uncertainty to which extent the findings can be extrapolated to the human condition.

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Acknowledgments

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* on behalf of WG1 of COST Action FA1402 – impARAS

ADVERSE OUTCOME PATHWAY (AOP)-BASED *IN VITRO* APPROACHES FOR THE EVALUATION AND PREDICTION OF THE SENSITISING POTENTIAL OF FOOD PROTEINS

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Before introducing proteins from new or alternative dietary sources into the market, a comprehensive risk assessment, including allergic sensitisation, should be carried out in order to ensure their safety. The working group 2 (*'In vitro* methods to predict sensitisation') of the European COST Action ImpARAS has recently proposed the adverse outcome pathway (AOP) concept to structure the current mechanistic understanding of the molecular and cellular pathways evidenced to drive IgE-mediated food allergies [1]. This AOP framework offers the biological context to identify *in vitro* approaches that reflect the molecular initiating and key events driving immune sensitisation to food proteins. As a part of the working group, we have clustered, structured, and discussed the existing *in vitro* models currently available, which have been previously used for allergenic food proteins, as well as the major read-outs, strengths, and limitations of these approaches.

The application of the AOP framework offers the opportunity to anchor existing testing methods to specific building blocks of the AOP for food sensitisation. In general, *in vitro* methods evaluating mechanisms involved in the innate immune response are easier to address than assays addressing the adaptive immune response due to the low precursor frequency of allergen-specific T and B cells. Novel *ex vivo* culture strategies may have the potential to become useful tools for investigating the sensitising potential of food proteins. When applied in the context of an integrated testing strategy, the *in vitro* methods identified by this working group may reduce, if not replace, current animal testing approaches.

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* on behalf of WG2 of COST Action FA1402 – impARAS

CAN YOU PREDICT A NOVEL ALLERGEN IN AN ANIMAL MODEL?

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Food allergy is a major health problem of growing concern. An increasing world population will require new protein sources for human nutrition. The introduction of new protein sources into the diet, such as novel proteins from insects or algae, or proteins processed by new techniques, may pose the risk for development of new allergies, either as a result of cross-reactivity with known food allergens or due to *de novo* sensitisation. Before market introduction an allergenicity risk assessment should be conducted, which calls for reliable, evidence-based and harmonised evaluation methods. Robust and reliable animal models for the identification and characterisation of potential allergens would be valuable tools for a safety assessment. *In vivo* assessment of the potential allergenicity of novel proteins provides a more holistic assessment than other methods available and may be the only viable method to evaluate the sensitising capacity. However, although various animal models have been proposed for this purpose, to date, none has been formally validated as predictive and none is currently suitable for testing the allergenic potential of new proteins. The design of animal models including the choice of animal species and strain, diet, route of administration, dose and formulation of the test proteins will greatly influence the outcome of the allergenicity assessment. Also, the choice of end-point parameters used for evaluating the allergenicity are highly critical for the interpretation of the outcome and the capability of determining the allergenic potency of novel food proteins. In the ImpARAS Cost Action project we extensively reviewed and examined important aspects of the design, conduct and interpretation of animal models for assessment of the allergenic potential of novel food proteins.

* on behalf of WG3 of COST Action FA1402 – impARAS

DEFINING THE TARGETS FOR THE ASSESSMENT OF IGE-MEDIATED ALLERGENICITY OF NEW OR MODIFIED FOOD PROTEINS

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The future of food innovation relies on the introduction of new or modified proteins into the diet. However, higher intake levels of novel proteins may inherently lead to health risks and could have the potential to cause novel IgE sensitisations and novel food allergies. Currently, the pre-market allergenicity assessment of these new sources relies on methods based on characteristics of known allergens, and only assesses potential allergenic hazards. However, there is no general consensus on the allergenicity parameters to use and the criteria that should actually be applied for the evaluation and decisions to be made during the risk assessment and risk management process. In this presentation, we propose that the most efficient and transparent strategy for allergenicity risk assessment of new or modified food proteins should be based on the decisions which risk managers need to take. Thus, the methodologies applied should be governed by the risk management questions to be answered.

An impARAS working group has generated an inventory of health outcome-related assessment parameters and potential criteria important for risk management decision-making. These possible parameters and criteria fall within hazard-based, exposure-based and risk-based categories and can be separated into the allergic IgE sensitisation phase or the elicitation of allergic symptoms phase. The impact of various options with regards to both method development and risk management practices was investigated. This first inventory and overview supports the decision-making process for desired targets, decision criteria and eventually preferred methods to better enable future allergenicity risk assessment and risk management of new or modified food proteins. Further development of this initial work within the framework of an international collaboration is crucial. Future research may provide more insights into why some proteins are more allergenic than others, and may increase the possibilities for quantitative risk assessment, but a clear outline of preferred decision-making criteria is needed from the risk management sector to help guide research during method development and ensure the applicability of newly developed methods for the questions at hand.

This work aims to promote discussions between different stakeholders on how allergenicity could be better defined for the purpose of safety assessment. Outcomes from these discussions and definitions could then be carried forward to better support and to provide targeted, uniform information to risk managers commissioned with the approval of new or modified protein-containing food products.

* on behalf of WG4 of COST Action FA1402 – impARAS

TUESDAY 2 APRIL 2019

**SESSION 3
RISK ASSESSMENT AND MANAGEMENT OF FOOD ALLERGENS – WHAT’S UP?**

By identifying risk factors during processing as well as determining appropriate ‘safe’ thresholds of allergenic proteins, the food industry can take increasingly proactive steps to avoid direct or cross-contamination as well as ensuring that their products are appropriately labelled and identified for those at risk. This session covers a range of critical topics in this area identifying both knowledge and data gaps.

NO TWO THE SAME: COMPARATIVE OVERVIEW ON ALLERGEN LABELLING REGULATIONS

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The lack of legislative harmonisation is one of the main obstacles for the international trade of food. Different countries have different labelling rules in place, different nutrition declarations and – when it comes to allergens – different lists of substances regulated, different exemptions and different positions about the so called ‘precautionary allergen labelling’ statements (PAL, e.g., ‘may contain...’). These factors create a highly complex legal environment for companies operating in several countries, hinder the creation of a common label for different systems and oblige companies to keep into consideration in their allergen management procedures a wider range of substances than the ones listed in their own country. For instance, if you are operating in Europe and marketing your products also in Japan or USA, you might have to consider as an additional allergen buckwheat (for Japan) or an extended list of tree nuts, including pine nuts or coconut (for USA).

It has to be remembered that a breach in allergens’ legislation will cause in most countries food recalls and – eventually – criminal prosecution, especially when consumers get injured. The presentation will offer a brief overview of such complexity, of the impact on day-to-day business operations and of the potential consequences for brand owners.

ANALYTICAL DETECTION METHODS VERSUS VITAL REFERENCE VALUES: WHAT ARE THE CHALLENGES AND SOLUTIONS?

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Public health officials and food manufacturers must determine how to protect the allergic population without limiting food choices and adversely affecting the quality of life of allergic individuals. Quantitative (probabilistic) risk assessment (QRA) provides the most robust approach to assess the potential risk associated with products that may contain unintended allergen residue. Such quantitative risk assessment modelling was utilised during the consideration and derivation of reference dose values that underpin the Allergen Bureau of Australia and New Zealand's Voluntary Incidental Trace Allergen Labelling (VITAL® 2.0) Program. Accurate exposure assessment, consisting of the concentration of unintended allergen residue and the quantity of the product that is consumed during an eating occasion, is an important component of the overall risk assessment and a number of variables must be carefully considered. The concentration of allergenic food residue (or protein from the allergenic source) can be determined by quantitative analysis of the ingredient or finished food product in question. It is critical to understand what the method is detecting and what units are reported (i.e. ppm commodity, total protein, or specific allergenic protein). Misinterpretation of the analytical results could clearly have significant effects on the overall risk evaluation. Additionally, the various analytical methods used for detection of potential allergen residues in food products must be sufficiently sensitive to support the use of programs such as VITAL® 2.0 or for potential consideration of regulatory action limits or reference doses. This presentation will examine the current sensitivity of analytical methods for detection of allergen residues and their ability to detect appropriate concentrations of allergen residue at various consumption quantities to support the VITAL® 2.0 reference doses.

THRESHOLDS AND COFACTORS IN ALLERGIC REACTIONS: LESSONS FROM THE TRACE STUDY

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Peanut allergy causes severe and fatal reactions. Current food allergen labelling does a poor job of balancing these risks against the burden of restricting food choice for allergic individuals because of limited data on thresholds of reactivity and the influence of everyday factors.

In the Threshold Reactivity And Clinical Evaluation (TRACE) study, which was a multicentre crossover study performed in Cambridge and London, peanut threshold doses for a UK peanut allergic population were estimated and the effect of sleep deprivation and exercise on these thresholds was examined. Peanut allergic participants were recruited from the general population. After confirmation of their peanut allergy by a baseline double blind placebo-controlled food challenge, participants underwent three open peanut challenges in random order: one with exercise following each dose, one with sleep deprivation preceding challenge, and one with no intervention. Primary outcome was the threshold dose triggering symptoms (mg of protein). Primary analysis estimated the difference between no-intervention challenge and each intervention in log threshold (expressed as percentage change). As a secondary outcome, dose distributions were modelled deriving eliciting doses in the peanut allergic population. Baseline challenges were performed in 126 subjects, 100 were randomised and 81 (mean age 25 years) completed at least one further challenge. The mean (SD) threshold was 214 mg (330 mg) for no-intervention challenges and this was reduced by 45% (95% confidence interval, 21-61; $P=0.001$) and 45% (22-62; $P=0.001$) for exercise and sleep deprivation, respectively. Mean (95% confidence interval) eliciting doses for 1, 5 and 10% of the population during baseline challenge ($n=126$) were 1.3 mg (0.8-2.0), 3.8 mg (2.4-5.7) and 7.0 mg of peanut protein (4.5-10.5), respectively.

In conclusion, exercise and sleep deprivation each significantly reduce the threshold of reactivity in people with peanut allergy, putting them at greater risk of a reaction. Incorporating these data to produce more evidence-based reference doses in allergen risk management will lead to more accurate food labelling which is critical for optimal protection of peanut-allergic consumers.

Acknowledgements.

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ACCEPTING AND COMPARING RISKS – RISK ASSESSMENT AND RISK MANAGEMENT DECISION CASES IN SWEDEN: A CASE STUDY

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The concept of risk management at the authority level is described in article 3.12 in the EU Regulation (EC) No 178/2002: "Risk management means the process, distinct from risk assessment, of weighing policy alternatives in consultation with interested parties, considering risk assessment and other legitimate factors, and, if need be, selecting appropriate prevention and control options." In Sweden, and other countries, studies show that undeclared allergens are a common cause of unexpected allergic reactions. Self-reported data show that 400/10,000 adults annually experience unexpected allergic reactions to food and data from the medical care shows that 3.2/10,000 children annually experience anaphylactic reactions to food. The current risk for unexpected allergic reactions to food within the total population could therefore be considered as unacceptable.

The target groups for the work of the Swedish National Food Agency (Swedish NFA) are different control authorities, food business operators, the public as well as the medical care. Examples of risk management measures are food legislation (e.g., labelling, thresholds), guidance for the food control, as well as information and advice to the public and to food business operators. Whether a certain risk level can be accepted, how the risk managers shall deal with uncertainties within the risk assessment and whether the measures will be proportionate and effective are certain aspects that need to be considered within the process of risk management at Swedish NFA. A risk could be accepted if the cost of reducing the risk would exceed the costs saved, or if the benefit outweigh the risk. During the presentation such comparisons regarding unexpected allergic reactions and other risks will be discussed.

There are always uncertainties within risk assessments and these should be clearly documented and presented to the risk managers. The risk managers need to consider these and other relevant factors to decide on whether to take risk reducing measures or not. A decision not to take measures or, e.g., wait for further research, is an active decision to accept the current level of risk. Risk management measures need to be proportionate to the level of risk. When dealing with toxicological and microbiological risks, thresholds or other measures are sometimes set in order to be 'as low as reasonably achievable' (ALARA) or giving an 'appropriate level of protection' (ALOP). Sensitive consumers group will not always be protected by these thresholds. Therefore, complementing consumer advice to the sensitive consumers need to complement the regulations. Thresholds for food allergens will alone not be the 'final solution' in order to decrease the number of unexpected allergic reactions. However, an acceptable level of protection for allergic consumers might not be reached without thresholds. Indeed, the most important conclusion from risk assessments of food allergens so far is that allergic consumers are more or less sensitive. Thus, the risk managers need to address this in its risk management process, to be able to decide appropriate risk reducing measures.

A QUANTITATIVE RISK-BASED APPROACH TO FOOD ALLERGEN MANAGEMENT

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Food allergy is a recognised public health issue across the world. However, whilst legislation covering allergens used as ingredients is now in place in many countries, allergens present unintentionally in products, e.g., through cross-contact during manufacturing, are only regulated in a few.

This talk will describe the current situation and challenges associated with the risk assessment and risk management of allergens present unintentionally in food products and use of precautionary allergen labelling (PAL). It will also cover how this could be addressed through a quantitative risk-based approach to allergen management, the latest research and activities in this area and the FoodDrinkEurope (FDE) recommendations on PAL.

ASSESSING THE EFFICACY OF CLEANING PROGRAMMES TO PREVENT ALLERGEN CROSS-CONTACT

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Cleaning of shared processing equipment is essential for reducing the risk of allergen cross-contact. In general, cleaning protocols are classified as wet, dry or a combination of both methods. The choice and effectiveness of cleaning protocols for removing allergenic food soils depend on the chemical/physical properties of the food allergens, the surfaces to be cleaned and other factors. Analytical tools for evaluating the effectiveness of allergen cleaning programs including total protein analyses/swabs, immunochemical approaches (ELISA; lateral flow devices), and DNA-based detection methods. The choice of the method used depends on the purpose of the test, the food matrix, the extent and manner in which the food is processed, the cleaning protocol used, the turn-around time, portability and cost. Methods used to assess the effectiveness of cleaning procedures must be validated before they can be used with confidence.

This presentation will examine the approaches, considerations and challenges associated with removing allergenic food residues from food-contact surfaces using wet and dry cleaning procedures and will discuss the analytical methods used for determining cleaning treatment effectiveness.

A RETAILER'S VIEW ON FOOD ALLERGEN MANAGEMENT

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This presentation is about a retailer's perspective on allergen management. How does Albert Heijn, as a retailer and, therefore, as a link between the food producers (and the supply chain) and the consumers, handle allergen management and allergen communication to its customers. Albert Heijn's role and responsibility will be emphasised, and the retailer's policy and requirements to the suppliers will be explained. I will conclude with a call for better anchorage in legislation and standards.

THRESHOLDS, FOOD INTAKE, SEVERITY, PARTICULATES, SAMPLING AND ANALYTICAL UNCERTAINTIES: CAN WE ACCOUNT FOR ALL ASPECTS IN RISK ASSESSMENT 2.0?

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To start with a question: is it relevant to include all these variables if we only do deterministic risk assessment, where all the input variables are reduced to two numbers – likely dose of allergen consumed and reference dose? With this in mind, there are of course areas where improvement of data will also benefit the deterministic risk assessment in its current form.

Thresholds and severity. At present, the reference doses most widely used are the ones calculated for the VITAL system. In theory, it is possible to use other challenge data sets to calculate (other) reference doses. The credibility of challenge data distributions and reference doses relies on consensus on the criteria used when including challenge data. Currently, the reference doses for the majority of foods are based on the low confidence interval of the ED05, and for some foods the current database is not good enough to establish a reference dose. The more challenge data added, and the more diverse the source of the data are, the more robust and trustworthy the resulting eliciting doses are. Understanding the consequences of using reference doses would increase, if challenge results forming the basis for the low end of the dose response curve were known, i.e., what was the severity of symptoms at low challenge doses? For risk assessment 2.0 we need to develop a system where challenge data can be added, curated and combined in a global dataset used for calculating reference doses and for probabilistic risk assessment.

Food intake. We have detailed data on food consumption on a meal level. This makes it possible to use specific consumption data for a food group [1]. For the deterministic approach, using the 75-percentile of the consumption will give an estimate on the safe side [2]. The whole distribution of consumption of a food group can be used in the probabilistic assessment. There is no reason to assume that, when a food allergic consumer chooses to eat a food, the portion sizes differs from non-allergic consumers.

Particulates. Traditionally, risk assessment is performed based on the assumption that the contamination is non-particulate. It is possible to estimate the probability of allergic reactions caused by particulate contamination. The overall probability will consist of several independent probabilities: the probability that a particle will be present in the product; the probability that a particle, or particles, would cause a reaction if ingested by an allergic consumer; and the probability that the product containing the particle(s) will be eaten by an allergic consumer (R. Crevel, unpublished).

Sampling and analytical uncertainties. In other areas, e.g., microbiology, there are detailed instructions on how samples should be taken. It would be a step forward if guidelines on sampling could be developed as well as guidelines on analytical methods to be used for different matrices. To benefit from more detailed data giving more than one number, the deterministic method needs to be improved, if possible, to allow for the uneven distribution of contamination.

Probabilistic risk assessment 2.0. The probabilistic risk assessment is based on distributions, e.g., of food consumption, contamination levels, challenge data, and prevalence of allergic persons in the population. We already have good data on consumption. If the knowledge of distribution of contamination is increased, it will be possible to create good distribution models of intake. It will be difficult to get the full use of these data without also having the distribution of challenge data. At present, these distributions are not shared, one of the reasons being confidentiality. Based on the aforementioned curated and combined global dataset, distribution could be calculated and shared as mathematical formulas, enabling the spread of knowledge without sharing confidential data.

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EARLY INTRODUCTION OF FOODS TO PREVENT FOOD ALLERGY

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The current strategies in preventing IgE mediated food allergies have drastically changed compared to before 2008. Until then, avoidance was the key strategy. The current hypothesis is that primary sensitisation to food allergens mainly occurs through the (non-intact) skin, especially in infants with eczema. Early gastro-intestinal exposure of allergens reduces the risk of sensitisation and, as several intervention studies have shown, reduces the risk of developing food allergy. Intervention studies have shown a reduced incidence (up to 80% reduction for peanut) of peanut allergy and egg allergy by early oral exposure to the allergen, especially in those infants at high risk for food allergy. The practical implication of these observations raises international questions about the safest and most effective strategy to achieve early introduction in a large group of infants at risk for food allergy. In the Netherlands, paediatric allergologists developed a guideline and instructions for health care professionals and parents focused on early introduction of high allergenic foods in infants at home. Implementation is encouraged by many educational sessions throughout the country.

The role of early gastro-intestinal exposure of allergens in developing tolerance may not be limited to prevention of food allergy. Oral immunotherapy for food allergy has not proven to be effective but is mainly studied in children above the age of 4 years old. The findings of studies on early introduction and a small study on oral immunotherapy in infants suggest that oral immunotherapy in young children may cause lifelong tolerance. Details of a recently started study in the Isala Hospital Deventer will be presented.

MICROBIOME MODULATION AS A TOOL TO MANAGE ALLERGIC DISORDERS

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Our body is attacked continuously by many different danger signals. An effective immune system is essential in order to protect in a balanced and resilient way. Unbalanced immune reactivity seems to play a key role in non-communicable diseases (NCDs) such as for example chronic pulmonary disease (COPD), allergies, asthma, diabetes, cancer and even cardiovascular diseases and obesity. According to the WHO these NCDs are not only relevant for high income countries but even for middle- and low-income areas. It is essential to develop new avenues to prevent the enormous increase in incidence and severity of NCDs including allergies. Early life programming including proper education of our immune system has been recognised as one of the most promising approaches.

The awareness of the importance of a diverse microbiome in immune-regulation/inflammation management and, as a consequence, impact on allergies is growing exponentially. After birth, the development of a 'healthy' gut microbiome is considered to play an important role in immune development and consequently inflammation management. Human milk is the golden standard early in life and very potent in creating a healthy microbiome and associated healthy immune system. Non-digestible prebiotic oligosaccharides and even some unique microbes are transferred by the mother through the breastmilk to the child. Some of these affect the composition and/or activity of the gastrointestinal microbiome leading to health benefits (prebiotic function). As an example, it has been shown that specific non-digestible oligosaccharides induce a gut microbiome comparable at least in part to breastfed infants. In addition to indirect effects on the immune system via microbiome changes prebiotic oligosaccharides can affect immune cells in a direct fashion as well. Many research groups are currently in search for the unique receptors, such as lectins/galectins, that are responsible for these direct immune effects. Recent clinical trials indicated that unique prebiotic fibres can impair the incidence and severity of allergic disorders. The majority of the studies so far indicated immune effects in individuals still having an immature immune system (infants and toddlers). However, very recent data indicated significant impact on other immune related diseases in other life stages as well, such as allergic asthma at an adult age.

Both pharma as well as specialised nutrition companies do see the highly relevance of microbiome manipulation for both prevention as well as treatment of immune related disorders such as food allergy. However more research, multicentre trials, and long-term follow-up studies are needed in order to validate the uniqueness of microbiome management in both classical pharma approaches as well as specialised and medical nutrition aimed at allergy management.

PREVENTION OF FOOD ALLERGIC REACTIONS – THE CLINICAL PERSPECTIVE

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Pollen-related food allergy is the most common food allergy in Northern and Central Europe. Up to 90% of pollen-sensitised individuals are allergic to foods that cross-react with pollen. Pollen-related food allergy is generally characterised by rapid onset of oropharyngeal symptoms, however, systemic reactions may occur. Frequently involved foods include *Rosaceae* fruits (e.g., apple, peach, cherry), *Apiaceae* vegetables (e.g. carrot, celery), peanut, tree nuts, and soybean. Because patients usually react to a many different foods, the food allergy has a high impact on daily life. The 'Learning Early About Peanut' (LEAP) study showed that peanut allergy could be prevented by introducing peanut at an early age into the diet. Since pollen-related food allergy is a secondary food allergy primarily caused by sensitisation to pollen, early introduction will not be effective in the prevention of this type of food allergy. So, other strategies are needed to prevent food allergic reactions in patients with a pollen-related food allergy. Examples of such strategies are heat processing, oral immunotherapy with food and consumption of low allergenic cultivars.

Clinical guidelines describe that heat processing food can reduce pollen-related food allergy symptoms, because major food allergens cross-reacting with tree pollen are heat labile. Clinical studies that have studied this are scarce. The effect of heating on clinical presentation of pollen-related food allergy has mainly been investigated for hazelnut and celery, and was found to eradicate symptoms in 15-71% of hazelnut and around 46% of celery allergic subjects. Furthermore, roasting of hazelnut resulted in higher dose thresholds and boiling of celery caused fewer moderate to severe reactions. So, thermal processing of causative foods in patients with pollen-related food allergy likely reduces symptoms, but the effect size may depend on the food concerned. Research with regard to the effect of consumption of alternative hypoallergenic cultivars on clinical symptoms in patients with pollen-related food allergy has focused on apple, showing that Santana apple appears to cause significantly less severe reactions than Golden Delicious apple. Neither skin prick test nor measurement of sIgE seem to predict allergenicity of different apple cultivars as determined by food challenge. Oral immunotherapy for pollen-related food allergy, was also only investigated for apple. Oral immunotherapy with apple in subjects with pollen-related apple allergy may be effective, but regular consumption after completion of the study is likely necessary to maintain tolerance.

So, heat processing, hypoallergenic cultivars and oral immunotherapy may diminish or completely prevent allergic reactions in some but not all subjects with pollen-related food allergy.

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TUESDAY 2 APRIL 2019

**SESSION 4
HOW THE USE OF NEW ANALYTICAL TOOLS BY COMPETENT AUTHORITIES
WILL IMPACT ALLERGEN RISK MANAGEMENT**

Testing for food allergens is an expensive endeavour. Many companies that take allergen management seriously, spend a significant amount of their quality control budget on analysis for the presence of food allergens. And yet, typically only a few food allergens are tested for. Competent authorities have now moved towards a new technology which will look at a wide range of food allergens in a single test. Several groups are currently working to develop and validate such methods.

ESTABLISHING MEASUREMENT SYSTEMS FOR ACHIEVING COMPARABLE DATA IN RISK ASSESSMENT RELEVANT UNITS FOR THE PRECAUTIONARY LABELLING OF FOOD

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Provisions of the food information to consumers legislation (Regulation (EU) No 1169/2011) address the requirement for the labelling of 14 priority allergenic ingredients to guide allergen sufferers in making informed choices. Precautionary allergen labelling may be used on a voluntary basis to inform on the likely unintentional presence of these substances. This 'may contain' labelling should only be used in conjunction with a quantitative risk assessment based on clinically validated thresholds for the content of the total food allergen protein in the final food product. Therefore, corresponding reliable and comparable data on allergens in food products have to be available. Recently, a common reporting unit for expressing measurement results has been agreed which is required for linking clinical thresholds, risk assessment and measurement results on the allergen content in foodstuff.

This presentation will provide the introduction to a concept for achieving measurement results for 'mg total allergenic ingredient protein per kg food' in a metrologically traceable, i.e., comparable manner. The corresponding measurement systems and the required anchor points and processes, in particular reference methods, certified reference materials and science-based conversion factors, will be explained. A pathway for establishing international comparability of measurement results on food allergen will be sketched.

FOOD SAFETY AND FOOD AUTHENTICITY BY PEPTIDE MASS SPECTROMETRY – CONSTITUTION OF A NEW § 64 LFGB WORKING GROUP FOR METHOD VALIDATION AND STANDARDISATION

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The monitoring of food allergens is a crucial task for consumer protection. In Annex II of Regulation (EU) No 1169/2011, 12 groups of materials causing most intolerances or allergies in food are listed that have to be labelled on products for the final consumer. Nowadays, testing for food allergens is mostly done by ELISA or PCR. While these technologies have proven useful over the past years, they also have some limitations and drawbacks. ELISA methods often have specificity issues whereas in PCR methods, allergen detection is achieved only indirectly via DNA. Moreover, both technologies have limitations in terms of the number of allergens that can be analysed in the same sample in one assay. For ELISA, typically only one allergen can be analysed, whereas for PCR, most assays range between one and four allergens.

In recent years, new methods for allergen detection have been developed utilising liquid chromatography coupled to mass spectrometry. Since most food allergies are caused by allergenic proteins, the new methods have the potential to detect not only the allergenic ingredient but also the specific protein that causes an allergy. Another major advantage of such methods is the detection of a multitude of allergen-causing proteins in a single analysis. At present, however, these methods generally lack validation, let alone standardisation. The Federal Office of Consumer Protection and Food Safety (BVL) in Germany has recognised the potential of such novel technology applications and therefore constituted a working group with the aim to identify appropriate technologies and validate these for official food control. The new working group, consisting of experts from the field, will focus mainly on the validation and standardisation of methods for allergen detection and food authenticity. The presentation will provide an overview over the current and future work of the newly-formed working group.

EVALUATION OF PERFORMANCE CRITERIA FOR ALLERGEN MEASUREMENT BY ELISA

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Before setting any performance criteria, we need to specify how to determine a performance characteristic. Relevant sources are EURACHEM guidelines, allergen-specific AOAC appendices and guidelines, allergen-specific CEN and ISO standards or specification and EU regulations (2002/657/EC and (EU) 519/2014). The most important characteristics are LoD, LoQ, specificity, interferences, precision, recovery/trueness, robustness, and stability which are characterised during a validation study. A test kit manufacturer will use the outcomes of these characteristics to test against internal or external criteria. The latter can be found in AOAC standard method performance requirements, in CEN and OIV documents or criteria directly submitted by potential customers of these test kits.

In case of allergens, the evaluation of performance characteristics is always a compromise due to the nearly unlimited number of possible matrices. It is furthermore complicated by the fact that different guidelines will describe different ways of, e.g., estimate an LoD or determine an LoQ. Especially the calculation of concentrations below LoQ for an LoD estimation is challenging and not standardised at all. We are often more and more confronted with questions from customers accredited to ISO 17025 of how to validate a matrix that is not in the scope of the method and if there is a difference to verification of a characteristic. Another important issue is the question of acceptance criteria for an ELISA run. A short view on the validation of qualitative test systems will also be given. The presentation will close with open questions for discussion by the auditorium regarding measurement uncertainty, the usefulness of an LoD, precision profiles, and the intended use of a method that collaboratively tested.

QUANTITATIVE ASSESSMENT AND TESTING, AN INDUSTRY PERSPECTIVE

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In the 17 years since the publication of the work of ILSI EU on allergen thresholds [1], there has been a substantial body of work to derive reference doses for allergens, including recent work to corroborate such reference doses via single dose challenge studies [2]. Significant work continues, such as the ILSI NA activity, to gather a US-specific set of challenge data on peanut for dose-distribution modelling. Knowledge on reference doses has reached sufficient maturity that they are increasingly being used by food businesses within the context of allergen management across their supply chains. This is reflected in important reference documents including the EU food industry guidance document which incorporates risk assessment [3]. Furthermore, the draft Code of Practice on Food Allergen Management for Food Business Operators [4] being developed by the FAO/WHO food standards programme Codex, states that precautionary labelling should only be used if 'the allergen may be present at levels that, based on an assessment of risk, could result in adverse consequences to the majority of allergic consumers'. Despite the large body of work on the derivation of reference doses, there has been little work on standardizing the application of such tools within the context of allergen management by food businesses, with the exception of the VITAL program of the Australian Allergen Bureau [5]. In the presentation we will explore how quantitative risk assessment can be used within the framework of allergen management, and how that should feed decisions on the use of precautionary labelling.

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REVIEW OF SUITABILITY OF ANALYTICAL METHODS FOR MEASURING ACTION LEVELS DETERMINED BY VARIOUS GUIDELINES

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Reliable analytical methods are a pivotal requirement for the introduction and adoption of reference doses in the European Union and beyond. The ILSI Europe Food Allergy Task Force has established an expert group to review the suitability of current analytical methods for measuring VITAL (Voluntary Incidental Trace Allergen Labelling) 2.0® reference doses for EU allergens in foodstuffs. This activity will report on the suitability of current analytical methods to reliably measure proposed allergens at various concentrations resulting from the use of the VITAL® 2.0 reference doses. Hence, the expert group has reviewed the suitability of quantitative analytical methods to reliably measure the presence of allergens at concentrations resulting from the use of the VITAL® 2.0 reference doses in relevant food matrices and depending on different serving sizes. This has especially included method sensitivity, specificity, and quantitative determination with regard to food matrix.

The review has been focused on currently applied methods such as protein-based enzyme-linked immunosorbent assay (ELISA) and mass spectrometry (MS), as well as DNA-based polymerase chain reaction (PCR). Methods for the detection of peanuts, soybean, hazelnut, wheat, cow's milk, and hen's egg were reviewed in detail. Additional allergenic foods with available VITAL® 2.0 reference doses were depicted in more detail if available methods complied with the main review criteria. Adequately sensitive ELISA, MS and PCR methods, that have specific advantages and limitations, were identified. A need remains to determine and limit factors of uncertainty with regard to obtaining comparable quantification of allergenic foods. The data obtained by the ILSI Europe review of the suitability of current analytical methods for measuring VITAL (Voluntary Incidental Trace Allergen Labelling) 2.0® reference doses for EU allergens in foodstuffs can be used and compared with action levels by various guidelines. Examples will be given to elucidate the differences between various guidelines, which may result in differences of suitability of measuring action levels with the same methods available.

OVERVIEW OVER FOOD ALLERGEN METHODS IN RESPECT TO ALLERGEN RISK MANAGEMENT AND CERTIFICATION SCHEMES

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Historically, food allergens were (and still are) tested by ELISA and in some cases by PCR. These methods have proven reliable in the majority of cases, but as always, many methods come with caveats. With PCR, the major issue is that products containing little DNA (but high levels of allergenic protein) cannot be detected with appropriate sensitivity. ELISA, on the other hand, can perfectly detect with high sensitivity, however, typically only detects one possible allergen in a given sample. If several allergens have to be tested for, several ELISA need to be run which increases the cost. For this reason, competent authorities are now in the process of validating multi-allergen detection systems based on mass spectrometry. These systems allow the simultaneous detection of several food allergens, and several publications by now described the detection of all food allergens as listed in Annex II of Regulation (EU) No 1169/2011. In parallel, Codex Alimentarius started a working group which will ultimately lead to manufacturers being required to have more control over the production processes involving food allergens.

How these parallel developments will affect stakeholders in the food supply chain will be discussed in this presentation.

TUESDAY 2 APRIL 2019

**SESSION 5
CONSUMER ANALYTICAL DEVICES FOR FOOD ALLERGENS**

A new kind of testing devices has emerged, which place otherwise complicated laboratory testing into the hands of consumers. Devices are based on different analytical principles and include immunological tests, molecular imprinting and DNA-/RNA-based capturing methods. However, to make such devices suitable for untrained consumers, the procedures to analyse and read results have to be simple and fail-safe.

OVERVIEW OF CONSUMER ANALYTICAL DEVICES FOR GLUTEN AND ALLERGEN DETECTION

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This presentation will provide a comprehensive overview of immunochemical food allergen assays and detectors in the context of their user-friendliness, through their connection to smartphones. Smartphone-based analysis is centred around citizen science, putting analysis into the hands of the consumer. Food allergies represent a significant worldwide health concern and consumers should be able to analyse their foods, whenever and wherever they are, for allergen presence. Owing to the need for a scientific background, traditional laboratory-based detection methods are generally unsuitable for the consumer. Therefore, it is important to develop simple, safe, and rapid assays that can be linked with smartphones as detectors to improve user accessibility. Smartphones make excellent detection systems because of their cameras, embedded flash functions, portability, connectivity, and affordability. Therefore, this talk will summarise the potential to modernise traditional allergen detection methods by interfacing them with a smartphone readout system.

THE NIMA SENSOR: A CONSUMER DEVICE FOR THE DETECTION OF GLUTEN OR PEANUT ALLERGENS IN FOOD

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Nima enables people to be their healthiest selves by giving them the power to know what is in their food. Nima does this by creating people friendly products and sensing technologies, generating and sharing data, championing and supporting people who care about what they eat. For individuals with a gluten and/or peanut allergy, avoiding illness can at times be difficult due to undetermined amounts of allergens in foods. A portable consumer device for testing gluten or peanut levels in foods could aid in this problem.

The Nima platform consists of a one-time use capsule that contains our proprietary antibody-based chemistry, an electronic sensor, and an app that can be connected via bluetooth to your Nima Sensor.

Third party validation:

- **Gluten:** In a study with FARRP (Food Allergy Research & Resource Program), 13 gluten-free foods were prepared; each food was spiked on a weight to weight basis with gluten levels of 0, 5, 10, 20, 30, 40, and 100 ppm before processing or preparation. Unprocessed and processed foods were tested with the handheld gluten sensor and by two gluten-specific enzyme-linked immunosorbent assays (ELISAs) on the basis of the R5 and G12 monoclonal antibodies, respectively.
- **Peanut:** In one study, 29 different commercially available quality control and reference materials obtained from various accredited providers were tested in replicates of 6 using the device. An additional evaluation was performed using 10 food matrices spiked to concentrations of peanut ranging from 5 to 100 ppm, with the 0 ppm matrices used as negative controls.

The portable gluten detection device detected gluten in all food types at the 30 ppm addition level, failing to detect gluten in only 5 (6.4%) of 78 subsamples. At the 20 ppm addition level, the portable gluten detection device failed to detect gluten in one type of pasta but detected gluten residues in 63 (87.5%) of 72 other subsamples. The device was able to detect gluten at the 10 ppm addition level in 9 of the 13 food matrices (41 of 54 subsamples, 75.9%). We conclude that the portable, handheld Nima gluten sensor functions reliably detect gluten residues at appropriate levels in a range of different foods [1]. For peanut, the first study yielded an average accuracy of 98.7% ($\pm 1.8\%$ CL) at the device LOD of 10 ppm, and above. The average True Positive Rate (TPR) was 98.9% ($\pm 2.2\%$ CL). For the second study, accuracy was 99.2% ($\pm 1.1\%$ CL) and the TPR was 100% for approved samples. The device correctly identified samples spiked to 10 ppm and above that had also been identified with ELISA testing as containing peanut above the LOQ of the assay.

In conclusion, independent validation of the gluten and peanut sensor devices indicate that the devices have the potential to aid sensitive individuals in their daily food choices.

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EVALUATION OF THE NIMA™ SENSOR FOR DETECTION OF GLUTEN RESIDUE IN INCURRED MATRICES

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The Nima™ sensor is now available for consumer use to determine of the presence or absence of gluten in food products. Foods labelled as gluten-free should contain no more than 20 ppm of gluten according to EU and US regulations. Analysis of food products incurred with known concentrations of gluten was conducted to determine the reliability of the sensor for detection of gluten in processed food products. Thirteen gluten-free foods (muffins, three different types of bread, three different types of pasta, puffed corn snack, ice cream, meatballs, vinegar and oil salad dressing, oatmeal, and dark chocolate) were prepared. Each food was spiked on a weight to weight basis with gluten levels of 0, 5, 10, 20, 30, 40, and 100 ppm before processing or preparation of the finished food product. Unprocessed and processed foods were tested with the Nima™ sensor and by two gluten-specific commercial ELISAs that utilise the R5 and G12 monoclonal antibodies, respectively.

The Nima™ sensor detected gluten in all food types at the 30 ppm addition level, failing to detect gluten in only 5 (6.4%) of 78 subsamples. The Nima™ sensor failed to detect gluten in one type of pasta but detected gluten residues in 63 (87.5%) of 72 other subsamples that were incurred with 20 ppm gluten. The Nima™ sensor was able to detect gluten in 9 of the 13 food matrices (41 of 54 subsamples, 75.9%) incurred with 10 ppm gluten but not in the three types of pasta and the puffed corn snack. The Nima™ sensor did not perform reliably at the 5 ppm addition level in 11 of 13 food matrices (exceptions: ice cream and muffins). In contrast, the commercial ELISA methods were highly reliable at detection of gluten residue in all the incurred food matrices at concentrations >10 ppm. The Nima™ sensor yielded a low percentage of false-positive results (4 of 111, 3.6%) in these food matrices. Thus, the Nima™ sensor performed reliably in the detection of gluten in foods having ≥20 ppm of incurred gluten with only 18 (5.9%) of 306 failures, if results of the one type of pasta are excluded. The Nima™ sensor worked with greater reliability as the gluten levels in the foods increased.

WEDNESDAY 3 APRIL 2019

**SESSION 6
FOOD ALLERGY CONTROL AND PREVENTION – A QUANTUM LEAP FORWARD**

With the advent of new knowledge and technologies, a new era is commencing. What are the challenges and opportunities for treatment and prevention of food allergy?

THE IMPACT OF IMMUNE INTERVENTIONS: A SYSTEMS BIOLOGY STRATEGY FOR PREDICTING ADVERSE AND BENEFICIAL IMMUNE EFFECTS

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Despite scientific advances it remains difficult to predict the risk and benefit balance of immune interventions. Since a few years, network models have been built based on comprehensive datasets at multiple molecular/cellular levels (genes, gene products, metabolic intermediates, macromolecules, cells) to illuminate functional and structural relationships. We used a systems biology approach to identify key immune pathways involved in immune health endpoints and rank crucial candidate biomarkers to predict adverse and beneficial effects of nutritional immune interventions.

Literature and database information was used to select the molecular and cellular dynamics involved in hypersensitivity, autoimmunity and resistance to infection and cancer resulting in a set of approx. 4,000 immune-related genes. An evaluation of the genes for each of the immune health endpoints was performed, which indicated that many genes played a role in multiple immune health endpoints, but also unique genes were observed for each immune health endpoint. This approach helped to build a screening/prediction tool which indicates the interaction of chemicals or food substances with immune health endpoint-related genes and suggests candidate biomarkers to evaluate risks and benefits. Several anti-cancer drugs and omega 3 fatty acids were evaluated as *in silico* test cases.

To conclude, we provide a systems biology approach to identify genes/molecules and their interaction with immune related disorders. Our examples illustrate that the prediction with our systems biology approach is promising and can be used to find both negatively and positively correlated interactions. This enables identification of candidate biomarkers to monitor safety and efficacy of therapeutic immune interventions.

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DEVELOPING A GENETIC RISK INDEX FOR PEANUT ALLERGY

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Over 200 single nucleotide polymorphisms (SNPs) have been found to be associated with food allergy (FA) in genome-wide association studies (GWAS). A Genetic Risk Score (GRS), is an index that can be derived from genome-wide association studies to summarise the genetic risk encompassed by a set of SNPs and is useful in risk stratification and prediction. Our objective was to use information from the Canadian Peanut Allergy Registry (CanPAR) GWAS study to develop a GRS and evaluate the positive predictive value of the GRS in CanPAR study.

The study aims to use the food allergy (FA)-associated SNPs using *P*-value thresholds ranging from 1.0×10^{-4} to 1.0×10^{-6} to generate a GRS using a weighted sum of the number of risk alleles (with values 0/1/2). Weighting each SNP by the natural log of their respective odds ratio (OR). We then evaluated the area under the curve (AUC) which is used to determine the effectiveness of the classification and the positive predictive value (PPV). The AUC value ranges from .5 to 1 with .5 being a poor classifier and 1 a perfect fit. A summary of results for each threshold level is given below.

In conclusion, we have demonstrated that with 336 SNPs we can achieve an AUC of .80, a threshold used for biomarkers. However, for medical diagnosis and treatment an AUC of .95 is desired.

<i>P</i> -value Threshold	# of SNPs selected			Statistical measures	
	Genotyped	Imputed	Total	AUC (95% CI)	PPV
1.00E-04	105	233	338	0.803 (0.78-0.82)	0.74
1.00E-05	18	51	69	0.694 (0.67-0.72)	0.62
1.00E-06	6	18	24	0.650 (0.63-0.68)	0.60

Acknowledgements.

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EPIGENETIC MODIFICATIONS – PROMISING TOOLS IN THE MANAGEMENT OF ALLERGIC DISEASES

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The analysis of epigenetic modifications including DNA methylation, post-translational histone modifications, nucleosome occupancy and small and long noncoding RNAs has attracted recently much interest in the field of allergic diseases. Epigenetics may indeed hold the key to explaining the high degree of plasticity of the immune response throughout life. Epigenetics may also mediate effects of environmental protective and risk factors on the development and the course of allergic diseases.

Recent research has provided evidence for an altered epigenomic landscape in disease-relevant cell populations. While still in early phase, epigenetic modifications, particularly DNA methylation and miRNAs, may have potential assisting in the stratification of patients for treatment and complement or replace in the future biochemical or clinical tests including potentially oral food challenges associated with substantial logistics for the clinician and some risk for the patient. First epigenetic biomarkers correlating with the successful outcome of immunotherapy have been reported and with personalised treatment options being rolled out epigenetic modifications might well play a role in monitoring or even predicting the response to tailored therapy. However, as many of the current studies have been performed on biological samples with heterogeneous cellular composition and epigenetic profiles are specific for each cell-type, results might be partially confounded by cell composition and further studies in larger cohorts with well-defined phenotypes in specific cell populations need to be performed prior to their implementation. Furthermore, the epigenome provides an interesting target for therapeutic intervention. miRNA mimics, inhibitors and antisense oligonucleotides are currently evaluated in clinical trials in other diseases, while extracellular vesicles as well as epigenetic editing represent future tools for the modulation of the cellular phenotype and responses. Moreover, interactions between the host epigenome and the microbiome are increasingly recognised and interventions of the microbiome could contribute to the modulation of the epigenome with a potential impact on the overall goal of prevention of allergic diseases.

THE FIRST REFERENCE GENOME SEQUENCE FOR BREAD WHEAT AND ITS IMPLICATIONS FOR WHEAT ALLERGY AND INTOLERANCE

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Wheat is one of the most important crops for human nutrition, but its components are also causative for several human diseases and insensitivities, such as celiac disease. With the recently published whole genome sequence for bread wheat [1], an important resource is now available to identify and study gene families related to wheat insensitivities and their products in a genome-wide manner.

Its complex genetic setup and large genome size of five times the human genome has made the wheat genome impossible to decode for decades. In 2005, the International Wheat Genome Sequencing Consortium (IWGSC; <https://www.wheatgenome.org/>) teamed up with the goal to produce a reference genome sequence for bread wheat by the year 2020. Thanks to novel algorithms and bioinformatics strategies, a reference genome sequence along with gene predictions could now be reported even earlier [1]. This new resource established the foundation for in-depth analyses of gene expression and regulation in the bread wheat genome [2] as well as the first genome-wide map of genes and gene families encoding immunoresponsive proteins [3]. This map is a first step to enhancing breeding efforts of wheat lines that are safer for human consumption and provide a higher environmental stability. Ongoing wheat pan-genomics and resequencing efforts such as the 10+ wheat project (<http://www.10wheatgenomes.com>) will greatly contribute towards understanding natural variation and the possible impact of different wheat genotypes on the immunoreactive potential of wheat products.

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PATHS TO ENGINEERING PEANUT FOR REDUCED ALLERGENICITY

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Peanut, *Arachis hypogaea*, is a crop of global importance, grown in subtropical and tropical regions of the world, but a part of diets in many countries where confectionery snack foods, peanut butter, and oils are consumed. Peanut is an unusual crop in its reproductive biology – it flowers above ground but sets its fruit underground. Exposure of fruits and seeds for a prolonged period during development may have contributed to the evolution of allergen isoforms as defence proteins in addition to other cellular functions. Sixteen peanut allergens are officially recognised (www.allergen.org), Ara h 1-3, 5-17, and encompass 7S and 11S globulins (Ara h 1 and 3), conglutins (Ara h 2, 6 and 7), a profilin (Ara h 5), a pathogenesis-related protein (Ara h 8), oleosins (Ara h 10, 11, 14, 15), a defensin (Ara h 12) and non-specific lipid transfer proteins (Ara h 9, 16, 17). The allergen landscape becomes even more complex in peanut because it is a tetraploid plant, hence it has at least two isoforms for each named allergen derived from the two progenitor genomes that were brought together during polyploidisation.

Of the named allergens, the globulins and conglutins are major seed storage proteins, functioning as a nutrient reservoir for seed germination. Given that Ara h 2 and 6 are the primary elicitors of anaphylaxis, we took a genetic approach to alter their expression levels through gene silencing and mutagenesis. RNA interference using an inverted repeat that targeted both Ara h 2 (100% sequence identity) and 6 (81% sequence identity) gene families successfully down-regulated expression of all isoforms, and in one transgenic line, Ara h 2 and 6 expression was essentially eliminated. Further analysis of these lines with LC-MS/MS label-free spectral counting confirmed the down-regulation of conglutins but revealed diverse collateral effects on other seed proteins/allergens such as increased Ara h 10 and Ara h 3-related and decreased Ara h 1-related. Seed storage protein alteration did not significantly affect morphology or vitality of seeds. While Ara h 2 and 6 silencing was stable across multiple generations in both greenhouse and field experiments, silencing is based on a post-transcriptional mechanism that could break down; therefore, a safer long-term approach would be mutagenesis. Prior to the advent of CRISPR-Cas9 gene editing in plants, we achieved knock out mutations for specific isoforms of Ara h 2 using random mutagenesis. Creating, identifying, and combining randomly generated mutations in all copies of Ara h 2 and Ara h 6 would be a lengthy endeavour, however.

The efficiencies of gene targeting in plants with CRISPR-Cas9 have reached a high level and hold promise for simultaneously creating knock outs of multiple allergen genes in peanut. Ara h 2 and 6 will remain primary targets because of their allergenicity and apparent dispensability to the plant. Creating a non-allergenic peanut is not feasible due to the large number of allergenic proteins with essential functions for the seed. Furthermore, the industry is not unified in how to handle an allergen-modified peanut given the high risk of mixture with wild-type peanut.

FOOD ALLERGY PREVENTION AND TREATMENT BY TARGETED NUTRITION

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In view of the dramatic rise in the prevalence of food allergy globally, effective *prevention* strategies have become a public health priority. Several models have emerged around the aetiology of food allergy, including the hygiene hypothesis, dual allergen exposure hypothesis, and vitamin D hypothesis. These form the basis for current and potential prevention strategies. Breastfeeding remains a key pillar of primary allergy prevention. Maternal elimination diets during pregnancy and lactation are not recommended for food allergy prevention. In recent years, there has been a shift away from prolonged food allergen avoidance to the proactive allergen introduction from 4 months of age. This approach is supported by two pivotal randomised clinical trials (LEAP and EAT studies) showing that the early introduction of peanut and other food allergens significantly reduces the risk of food allergy. However, the implementation of this strategy at the population level still raises logistic problems, including patient selection and development of suitable food formats for young infants. Other prevention strategies, including vitamin D supplementation, are currently under evaluation.

The *treatment* of food allergies has also seen major transformations. While strict allergen avoidance is still the key treatment principle, there is a greater focus on desensitisation and tolerance induction by oral and epicutaneous immunotherapy. In addition, specialised hypoallergenic infant formulas for the treatment of infants with cow's milk allergy have undergone reformulation, including the addition of lactose and human milk oligosaccharides in order to modulate the gut microbiome and promote early immune development. Further research is needed to inform the most effective food allergy prevention strategies at the population level. In addition, the wider application of food allergen immunotherapy may provide better health outcomes and improved quality of life for families affected by food allergies.

A REAL-WORLD USE OF DIGITAL LEDGERS FOR CONTROLLING CONSUMER ALLERGEN RISK: A CASE STUDY

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Allergen control remains a significant problem as rates of allergen sensitivity in human populations continues to rise. Whilst much work has been done in the food supply chain from a regulatory and enforcement perspective, the consumer awareness side of the problem has received far less attention. Recent cases of consumer deaths due to allergens have received much publicity and these look set to spark a new round of scrutiny on the rules on allergen control and labelling. Digital ledgers show much promise in the food sector being able to effectively communicate allergen information to consumers. The speaker will discuss current supply chain approaches to controlling allergens in food and demonstrate a real-world use case of how digital ledger technology can help with the consumer awareness and reduce the incidence and impact of allergen sensitivity.

WEDNESDAY 3 APRIL 2019

**FINAL PLENARY MEETING
A FOOD ALLERGY-FREE WORLD ON THE HORIZON**

What does the (near) future hold?

WHERE ARE WE IN 2025 IN PROTECTING EXISTING FOOD ALLERGY SUFFERERS?

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Over the last 20 years, several actions have been taken by various players – public health agencies, the clinical community, food regulators, the food industry and consumer organisations – with the aim to enhance the protection of food allergic consumers. Food recall statistics continue, however, to show that food allergens are high on the list of food hazards to which these recalls are attributed.

While a number of interventions have led to major progress, such as clearer food labels and improved food processing practices, we continue to aspire to a world, where food allergy incidents are a thing of the past. This presentation will review progress being considered to equip food allergy sufferers and their care takers with some of the tools that can enhance the effectiveness of prevention measures. Smart food labels supporting more and more personalised nutrition, integrated food processing practices with direct notification to concerned consumers, enhanced product and food processing certification, with a stronger emphasis on food allergen management are realistic measures that can be envisaged for implementation in the short term. Integrated food allergy prevention strategies encompassing some of these applications, along with curative approaches under development and close engagement with all stakeholder communities are likely to produce major progress towards a food allergy incident free environment by 2025.

WHERE ARE WE IN 2025 IN CURING FOOD ALLERGY

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No curative treatments for food allergy are available and patients have to adhere to avoidance diets. This does not prevent unexpected reactions, due to mistakes, inadequate labelling and unintended cross contamination. Therefore, there is a high need for curative treatment options. In recent years many studies addressed whether specific immunotherapy via different routes: e.g. oral, sublingual, intralymphatic, epicutaneous and rectal could be effective in treating food allergy. Many studies showed limited effectiveness or an unacceptable frequency or severity of side-effects. Research is rapidly expanding. Two immunotherapy options are within reach.

Novel developments. Both epicutaneous and oral immunotherapy were given FDA breakthrough status and are currently in their last phase of development and expected to become available late 2019. So far, the focus was on peanut allergy, and particularly in children. It can be expected that immunotherapy for milk and egg will follow and might be available around 2025. Still there is a high need for immunotherapeutic options for adults and for other important allergens like nuts.

Treatment options expected in 2025. Anti-IgE showed effectiveness on increasing eliciting doses and reducing side-effects during immunotherapy. More effective anti-IgE treatments are underway. Such treatments either as stand alone or in combination with specific immunotherapy will become available. For various atopic and related diseases, like atopic eczema, asthma and urticaria novel biologics, targeting cytokines and cytokine receptors in pathways that might also be involved in food allergy, are becoming increasingly available. Adjuvant strategies to reduce side-effects of immunotherapy are underway using pre- or probiotics, inducers of the Th1 and or regulatory T cell response like CpG or inhibitory oligodeoxynucleotides (particularly in adults). Mast cell inhibiting therapies are underway, which also might prove to be effective in inhibiting food allergic reactions, like rapamycin or dietary components, such as polydatin and resveratrol.

Curing food allergies beyond 2025. The field of (immuno)therapy will change rapidly in the coming years. More sophisticated therapies are under investigation. The concept of developing modified or hypoallergenic allergens with reduced allergenicity but retained immunogenicity has been proven. Another field explored, is immunotherapy using peptides of relevant (peanut) allergen (T cell) epitopes or DNA vaccines. This is promising news for food allergic patients and their health care givers, although further development probably requires years of additional research.

WHERE ARE WE IN 2025 IN PREVENTING FOOD ALLERGY?

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The last decade has seen a shift in one of the major food allergy prevention paradigms – the timing of introduction of potentially allergenic foods into infants' diet. With the 81 per cent relative reduction of peanut allergy in the introduction arm of the LEAP study, early dietary intervention (EDI) is the most powerful preventive strategy currently described [1]. EDI has proven to be effective for peanut and egg allergy prevention. The intervention is allergen specific and it remains to be shown whether it is equally effective for other common food allergens.

Like all new strategies, EDI brings challenges with its implementation which will need to be faced in the coming years. Firstly, should EDI be applied to the whole population or high-risk population only? While the number needed to treat (NNT) is as low as 8 (7-23) for peanut allergy prevention in children with moderate to severe eczema, it reaches 56 (45-167) in the general population [2]. Due to the narrow window of opportunity in terms of age the intervention should occur, efficient selection strategies are needed for EDI to be effective. Dedicated early-introduction clinics are necessary to ensure safety at the first exposure to food in high risk children. On the other hand, wide-ranging training of the general health professionals is critical to enable implementation in the low risk group.

Moreover, the differences between the outcome of intention-to-treat analysis and per protocol analysis on the EAT study indicates the challenge of compliance to EDI requirements, especially if multiple foods are being introduced [3]. Simplifying the procedure by providing ready to use protein mix or limiting the number of introduced foods as well as efficient follow-up of high-risk patient seem to be necessary.

Finally, in light of the dual allergen exposure theory, the balance between food allergen exposure through skin and gut plays an important role in development of food sensitisation. Significant increasing trend has been noted in recent years in the prevalence of skin allergy. Therefore, strategies concentrating on enhancing skin barrier form a second pillar of food allergy prevention [4].

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Unprocessed cow's milk suppresses allergic symptoms in a murine model for food allergy – a potential role for epigenetics

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Epidemiological studies have shown an inverse relation between unprocessed cow's milk consumption and the development of asthma and allergies. This protective effect seemed to be abolished by milk processing. Previously, we confirmed the epidemiological findings on asthma by showing causality. In the present study, we investigated whether unprocessed cow's milk is also protective in a murine model for food allergy. Besides, we looked at possible changes in histone acetylation to investigate the involvement of epigenetic regulation. C3H/HeOJ mice were sensitised intragastrically (i.g.) once a week for five weeks with ovalbumin (OVA) using cholera toxin (CT) as an adjuvant (d0, 7, 14, 21, 28). Prior to sensitisation, mice were orally treated with unprocessed milk, processed milk or PBS (as control) for eight consecutive days (d-9 to -2). Five days after the last sensitisation (d33), mice were challenged intradermally (i.d.) in the ear with OVA to determine acute allergic symptoms. On the same day, mice were challenged i.g. with OVA. Eighteen hours after the i.g. challenge mice were killed and organs were obtained for *ex vivo* analysis (d34). In addition, epigenetic modifications in Th1-, Th2- and regulatory T cell-related genes of splenocyte derived CD4+ T cells were analysed after milk treatment (d-1) as well as at the end of the study (d34). OVA sensitised mice receiving unprocessed milk showed decreased allergic symptoms compared to sensitised mice receiving PBS. The acute allergic skin response and anaphylactic shock symptoms were reduced and the body temperature remained high. OVA-specific IgE levels were also decreased. These protective effects were not observed when sensitised mice received processed milk. Looking at epigenetic modifications, unprocessed milk exposure for eight days led to higher acetylation of Th1-, Th2- and regulatory T cell-related genes of CD4+ T cells compared to processed milk (d-1). At the end of the study (d34) this general immunostimulation was resolved and acetylation of Th2 genes was lower compared to processed milk. In conclusion, unprocessed cow's milk reduces allergic symptoms in a murine model for food allergy. This protective effect was not observed after exposure to processed milk. The general immunostimulation in the spleen after exposure to unprocessed milk could be responsible for the observed tolerance induction, suggesting that epigenetic mechanisms contribute to the allergy protective effect of unprocessed cow's milk.

P2

Household exposure to food allergens: a risk for sensitisation?

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Exposure to food allergens is a pre-requisite to the development of food allergy. It is not fully understood what levels of exposure to allergens or what route of exposure is most important for allergic sensitisation. Food allergens present within household dust and in the air may contribute to allergic sensitisation of individuals at risk of developing food allergies. We sought to determine the precise levels of specific food allergens within household dust, and measure levels of inhaled exposure using an innovative nasal filter. To determine which allergens were present, settled dust samples were collected from a range of households within Europe. Seven common allergens were simultaneously quantified using a highly sensitive multiplex array for allergens; peanut (Arah3 and Arah6), milk (Bosd5), egg (Gald2), hazelnut (Cora9), cashew (Anao3) and shrimp (tropomyosin). To determine whether aerosolised food allergens could be detected using the nasal filter method, they were worn in a variety of settings and compared to the standard IOM method using PTFE filters. Each of the allergens assessed were readily found within household dust. Major allergens from egg (Gald2) and milk (Bosd5) were found to be the most abundant, with levels as high as 275 µg allergen/g dust. The least abundant food allergen was Cora9. All seven allergens were also detected using the nasal filter method. This novel approach to air sampling proved to be more effective as, for every allergen, a higher fraction of samples was positive and detected at higher levels in comparison to the standard IOM method. In conclusion, food allergens within household dust are within the same range and higher as those known to cause sensitisation to common indoor allergens. Milk and egg are especially prominent exposures. These findings suggest that household dust may be an important source of food allergen sensitisation. Additionally, levels of aerosolised food allergen could also be detected and suggest sensitisation could occur through inhalation.

Implementation and optimisation of targeted LC-MS methods for simultaneous detection and quantification of major food allergens

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Liquid chromatography-mass spectrometry (LC-MS) is a well-known technology for high resolution and sensitive detection and quantitation of a broad scale of molecules. LC-MS can be applied in a non-targeted mode for discovery and molecular profiling of composition of a biological extract or in a targeted mode for very specific detection and quantitation of molecules. Targeted LC-MS (with multiple reaction monitoring (MRM)) has the potential for being a very powerful alternative to ELISA for allergen detection and quantitation. LC-MS/MRM yields high sensitivity and highly selective detection of multiple protein sequences in a single assay. The use of LC-MS/MRM allows targeted detection of various protein components (epitopes) responsible for human allergic responses. The number of epitopes in allergenic food products is much higher than the 14 product groups currently requiring food labelling. The main benefit of targeted LC-MS/MRM is simultaneous detection of multiple allergens in a single assay. These could be multiple allergens from the same product group (such as for various nuts), or a combination that encompasses different product groups (e.g., milk, egg, soybean, lupin). The application of LC-MS/MRM provides greater flexibility in terms of tailoring an assay to the required analysis. The method is not dependent on use of antibodies, and thereby less promiscuous. Detection may target common proteins on a very wide scale or may specifically target the presence of separate allergenic epitopes or isoform variants. We have investigated different aspects for the development of an efficient and practical analysis procedure for multiplexed detection of different allergen sources in diverse food matrices. The following aspects were addressed: proper target selection per allergen (group), protein extraction procedures, digestion conditions, LC-MS target method development, interference analysis, sensitivity and reproducibility testing. Further fine-tuning and streamlining of procedures will ensure a cost-effective implementation within the practices of food-screening laboratories. We present several findings of our method development on our poster.

P4

Application of real-time PCR for the simultaneous detection of tree nuts and peanut allergen in processed food

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The European food labelling Directive 2000/13/EC reports a list of allergenic ingredients: gluten-containing cereals, crustaceans, molluscs, fish, peanuts, soybeans, eggs, milk and dairy products (including lactose), nuts, celery, mustard, sesame seeds, lupine, sulphite and products thereof. To protect the health of consumers, the declaration of these ingredients has been made mandatory, regardless of their amounts in the final product. PCR-based methods amplifying specific DNA sequences can be a tool for detection of allergenic species. PCR is a convenient, fast and robust technique for food quality and safety control. We developed two assays for the simultaneous detection of cashew, hazelnut, pistachio, macadamia nut, peanut and almond, walnut, pecan nut and Brazil nut. These assays are based on molecular beacon technologies targeting species-specific multicopy gene enhancing sensitivity and outperforming, while complementing, ELISA tests results.

P5

Development of reference materials for allergens detection in food using digital droplet PCR

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Droplet digital PCR (ddPCR) is the newest generation of PCR techniques, based on compartmentalisation allowing single molecule detection. The basis of digital PCR is to quantify the absolute number of targets present in a sample, using limiting dilutions, PCR and Poisson statistics through partitioning of the reaction mix across a large number of partitions containing zero, one or more copies of the target nucleic acid. After end-point PCR amplification, each partition is scrutinised and defined as positive ('1', presence of PCR product) or negative ('0', absence of PCR product), hence the term 'digital'. In particular, droplet digital PCR (ddPCR) approach combines partitioning of the PCR test into several thousands or millions of individual droplets in a water-oil emulsion, with the use of flow cytometry to count positive PCR tests. It is less dependent of PCR inhibition and allows absolute quantification without the need of calibrators. Recently, a ddPCR commercial system has been demonstrated that enables suitable metrological performance in food and feed analysis procedures. We have developed a set of reference materials (RMs) for different allergenic targets based on ddPCR technologies. These RMs can be an innovative solution for quantification allergens in food matrix to increase the safety level of food traceability in agreement with the current and future legislation.

Food consumption amounts of food allergic patients resemble that of the general population for use in food allergen risk assessment

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Intake of unintended allergens in food products poses a risk for allergic patients. Estimation of that risk is possible with food allergen risk models, using consumption data available from general population surveys. The assumption in these risk models is that the amount consumed in these surveys is representative for the amount consumed by the allergic population. The objective of this study was to investigate whether food consumption amounts at single eating occasions in the allergic population are comparable to those in the general population for use in allergen risk assessment. In a cohort of food allergic adults of the University Medical Center Utrecht (n=73 patients), food consumption was recorded by 2 non-consecutive 24-hour recalls, comparable to the method used for the Dutch National Food Consumption Surveys for the general population. Products consumed were assigned to 55 food groups developed for food allergen risk assessment. The consumption distributions of the allergic and general population of the national survey (DNFCS 2007-2010) were statistically analysed for possible differences. Surveys were completed for peanut and/or tree nut allergic (n=35) and milk and/or egg allergic individuals (n= 38). For 94% of the food groups (30 out of 32 food groups available for analysis) in the milk and/or egg allergic population and for 90% of the food groups (24 out of 27 food groups) in the peanut and/or tree nut allergic patients, the amounts consumed did not statistically significantly differ from that in the general population. The food allergic population consumed a statistically significantly different amount per eating occasion for only 3 food groups, with following differences: 30% for food group oils and frying fats, 64% for crackers, and 110% for unprocessed fruit and vegetables. These differences did not lead to proportional differences in outcomes of probabilistic allergen risk assessment. Although food allergic individuals might choose an alternative product from the same food group, reflecting their allergy profile. Overall, food allergic patients consumed the vast majority of food groups in equal amounts as did the general population. In conclusion, the study outcome supports the hypothesis that for allergen risk assessment the food consumption amounts at single eating occasions in the allergic population do not differ significantly from those in the general population.

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QSight® 220 triple quadrupole mass spectrometry as a screening tool to detect traces of milk and egg allergens in incurred food matricesL. Monaci¹, E. De Angelis¹, R. Pilolli¹ and **Aristide Ganci**²

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Food allergens are a widespread and public health problem and its inclusion in foods should be clearly indicated in the respective food label as required by the legislation issued in Europe [1]. Beyond the voluntary addition of allergenic ingredients into foods to improve their characteristics, an additional risk of cross-contamination is likely to exist especially along the food chains where different foods are processed, especially if strict risk assessment plans are not put in place by the food industry [2]. To protect the health of allergic consumers from the inadvertent or intentional contamination by allergens, different analytical methods were devised over the years, mainly employing an antibody-based recognition although they suffered from some limitations especially in case of complex and processed food products. More recently, mass spectrometry methods have gained the primacy of being considered reliable and multi-target approaches for allergen identification, enabling identification of the contaminating allergens with the highest confidence through detection of specific and prototype signature peptides. Several methods have been developed over the last ten years, exploiting different mass analysers employing both low resolution and high-resolution systems [3] and harmonisation and validation of a MS-based method capable of detecting several allergens in one run is among the primary objectives of recent European projects and of the ThRAII project [4]. In this poster, we report for the first time the optimisation of a LC-MS/MS method that exploits the features of the Perkin Elmer triple quadrupole mass spectrometer QSight® 220 for milk and egg allergens detection in cookie, chosen as a representative complex food matrix, previously incurred with skimmed milk powder and whole egg. The analytical method was duly optimised, and all instrumental settings were thoroughly tuned aiming at maximising the signals of specific signature peptides along with their transitions. The best three fragments were identified and monitored along the chromatographic run (one quantifier and two qualifiers) for each allergenic ingredient in the chosen food. Finally, performances of the method were evaluated also by employing external reference standard peptides. The lowest amount detectable of standard peptide injected was as low as 10 pg both for egg and milk peptides, highlighting the excellent capabilities of the analytical platform in use. Calibration curves built in the incurred food matrix also enabled calculation of the final LODs in the incurred cookies that were in the lower few ppm level for each allergenic ingredient.

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High resolution mass spectrometry identification of peptide biomarkers for the development of a quantitative reference method: application to egg

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Food allergy is a rising global health problem. Strict avoidance of allergen-containing food products remains the best solution for allergic patients. To allow patients to protect themselves, the labelling of 14 ingredients that have the potential to cause an allergic reaction is required by EU legislation (Regulation (EU) 1169/2011). Unfortunately, incidental presence of allergens in food is also possible due to cross-contamination and this is not covered by legislation. The field requires the development of sensitive and robust allergen detection methods. Thanks to technical advances and numerous studies over the last few years, mass spectrometry (MS)-based methods became methods of choice for allergen detection. To improve the efficiency of food control, the 'Allersens' project aims to develop and validate a multi-allergen MS-based method (LC-MS/MS) targeting 4 priority allergens (peanut, hazelnut, egg and milk) in terms of prevalence and reaction severity. One important criterion is that the method must be robust to food processing. The first step of this project, as in the development of any MS-based method, is the identification of peptide biomarkers for the allergens. This objective was fulfilled with an empirical approach using high resolution mass spectrometry (HRMS). Several standardised test materials, representative for food products (heat treatment, low pH, fatty and complex matrices) and containing separately the 4 allergens, were prepared. These reference materials were analysed by HRMS. Identified peptides were then filtered according to several, strict criteria to identify the best performing peptide biomarkers in terms of specificity, sensitivity and robustness of the future quantitative method. Results obtained for the selection of egg peptide biomarkers will be presented and discussed.

Oral tolerance induction for cow's milk allergy prevention using polymeric nanoparticles loaded with beta-lactoglobulin derived synthetic peptides

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Food allergy is one of the first outcomes of allergic disease and 2-4% of infants are affected by cow's milk allergy (CMA), persisting in 20% of cases in adult life. An atopic status in early life increases the risk of developing allergic diseases later in life. Therefore, early intervention for CMA prevention is important. Oral tolerance is organised in the intestine, at the inductive sites (M-cell covered Peyer's Patches and mesenteric lymph nodes) where antigen presenting cells instruct the adaptive immune response. Recently, selected synthetic peptides (18-AAs) based on the sequence of β -lactoglobulin, a major allergen in cow's milk, were identified to facilitate oral tolerance induction [1,2.] To prevent early life CMA, we aim to encapsulate selected β -lactoglobulin-derived peptides (referred to as peptide 3 and 4) into PLGA nanoparticles (NPs). Encapsulation of the peptides aims not only to protect the orally administered peptides from gastro-intestinal digestion, but also to deliver the peptides across mucosal linings for effective induction of oral tolerance. The peptides 3 and 4 were encapsulated in PLGA NPs by double emulsion evaporation method. *In vivo*, 3-week old female C3H/HeOuj mice were given orally either PBS, whey protein, a mixture of free or PLGA encapsulated peptides 3 and 4, or free peptides 3 and 4 plus empty PLGA NPs for 6 consecutive days, followed by oral sensitisation with whey protein and cholera toxin weekly for 5 consecutive weeks. One week after the last sensitisation, the mice were intradermally (ear) and orally challenged with whey protein. The acute allergic skin response (ear swelling), anaphylactic shock score, and body temperature were measured as allergic outcome parameters. Peptide 3 and 4 were loaded in PLGA NPs with an encapsulation efficiency of ~70-90%, showing no burst release in PBS buffer (37°C, pH 7.4) from the polymer matrix. *In vivo*, the mice pre-treated with NPs loaded with peptides 3 and 4 showed a reduced acute allergic skin response compared to the group that was pre-treated with the free peptides ($P < 0.05$). The anaphylactic shock score and body temperature were in line with these results. In conclusion, the β -lactoglobulin-derived synthetic peptides 3 and 4 showed a high encapsulation efficiency in PLGA NPs. These NPs induced oral tolerance and largely prevented development of CMA symptoms to whole whey protein. This proof of principle study allows us to further develop the concept of peptide-loaded PLGA NPs for CMA prevention.

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The feasibility of real-time PCR as a tool for seafood allergen detection and quantification

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Fish and crustaceans, such as shrimps, crabs and lobsters, are some of the most common allergenic foods responsible for eliciting the majority of the seafood-allergic reactions. Despite their compulsory labelling [1], the allergic individuals can inadvertently consume the offending foods due to mislabelling or cross-contamination during processing. This emphasises the need of verifying the labelling compliance, which should rely on highly sensitive, accurate, specific and fast methods to enable the detection of trace levels of allergens in foods. In the present work, two real-time PCR approaches are proposed targeting the 16S rRNA mitochondrial region, as a universal marker for fish [2] and crustacean detection [3] in foods at trace levels. Sequences of the 16S rRNA mitochondrial gene were selected from NCBI database from a set of different crustacean (n=17) and fish (n=24) species, which were aligned with BioEdit version 7.2.5 software (Ibis Biosciences, Canada) using ClustalW (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) and suitable primers/probes were designed to amplify as much crustacean or fish species as possible. Model mixtures of fish in béchamel sauce and shrimp in béchamel sauce were prepared in the range of 50-0.0001% (w/w) for method development. Several crustacean (n=18), fish (n=26), molluscs, meat and plant species were used for specificity testing and samples of seafood products were analysed for assay applicability. Two real-time PCR approaches based on TaqMan probes were developed targeting, each of them, sequences of the 16S rRNA gene as universal markers for crustaceans or fish. Both systems showed similar relative sensitivities for fish or crustaceans in béchamel sauce (0.0001%), but the fish assay presented a higher absolute sensitivity (0.01 pg of DNA) than the crustacean assay (0.1 pg of DNA). The real-time PCR methods exhibited high performance for quantitative analysis in the range of 0.0001% to 50% as inferred by the calibration curve parameters, being effectively validated with blind mixtures. The assays were successfully applied to processed seafood samples, allowing estimating the crustacean or fish contents and verifying their labelled amounts and precautionary labelling [2,3]. The results suggested a great level of mislabelling and/or fraudulent practices, mainly in the case of fish products. The proposed real-time PCR methods proved to be useful tools for the accurate detection and quantification of crustacean or fish in foods down to trace levels, showing their feasibility for allergen detection or authenticity testing.

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P11

Proficiency-testing schemes for gluten detection in artificially contaminated food: comparative analysis between spiking levels and assigned values

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Food allergies are increasing worldwide and becoming a public health concern. Due to the importance of the detection and/or determination of allergens in food, the number of laboratories interested in these analyses has gradually increased in recent years. The main goal of these analyses is to ensure higher quality of allergen-free food, allowing the detection of allergens both during the different steps of production and in the final products. BIPEA organises regular proficiency-testing schemes (PTS) in many analytical domains, including the detection and quantification of allergens in food. However, the setting up of PTS for analysis of allergens is a challenge, since food allergens are more or less denatured mixtures of non-defined proteins in complex matrices and their quantification is intrinsically related to the food preparation techniques: for processed food the extraction of denatured or altered proteins tends, in fact, to be difficult, due to their reduced solubility as compared to native proteins. The objective of this work was to determine differences in the detection of gluten in artificially contaminated food, between baked matrices and non-baked ones. 8 PTS has been organised on infant flour and cakes samples spiked in gluten gathering an average of more than 20 laboratories. Infant flour samples were spiked with wheat flour in well controlled proportions and sent to the laboratories without any heating process. Conversely, cake samples were prepared including a defined gluten content in the recipe before the cooking process and then baked. For each test, a statistical treatment of the data was performed according to ISO 13528 and assigned (consensus) values were calculated from the participants' results obtained performing the enzyme linked immunosorbent assay (ELISA) technique. Assigned values were compared to the theoretical ones (spiking levels). Recovery rates of infant flour samples were around 100% for each performed test. Rather the contrary, recovery rates of processed cakes varied between 40% and 80%. These results confirm that thermal treatment is an important factor that can affect allergen recovery and detection and that the analytical procedures need to be optimised to better detect and quantify allergens in thermally processed foods.

The behaviour of allergenic food particles in food production and the risk of their unintended presence in products

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Allergenic food particles (typically ~1-5 mm) may unintentionally end up in food products during the production process and can pose a risk for the allergic consumer. A single particle can pose a high risk when ingested by an allergic consumer, as the dose of allergenic protein is relatively high compared to minimum eliciting doses for allergic symptoms. However, awareness around the actual risk due to allergenic particles in packaged foods may be lacking due to an abundance of caution regarding labelling by food producers and a lack of fundamental research regarding allergenic particle behaviours. It has always been assumed that the behaviour of particles in food production facilities is rather unpredictable due to differences in particle characteristics (e.g., size, hardness, shape) and various factory settings. However, studies with a variety of particles could provide insight into their fate during food production and the magnitude of resulting risks. We studied the behaviour of several types of particles encompassing a range of size, shape and density characteristics (e.g., mustard seeds, sesame seeds, decoration pearls, hazelnut pieces and walnut pieces) in laboratory and pilot production settings. The main behaviour characteristics studied were the bouncing height and spread around a point where the particles are handled in a factory setting. The laboratory experiments were coupled to experimental and theoretical physics data to verify the trajectory of particles when dropped and bounced. Experimental settings were varied to cover multiple production facility settings (e.g., moving or tilted production belt). Generally, it was found that the spread of particles was less than previously anticipated and limited to the direct environment of the application point. Furthermore, we observed that particle characteristics (i.e., size, shape, hardness) influence behaviour to at least some extent (i.e., spherical particles spread farther than non-uniform particle shapes). The combined results of the experiments, theory and TNO expertise resulted in guidance for food producers to assess, manage and reduce the risk of particle cross-contamination. The guidance provides checklists and example calculations (maximum bounce height and spread) for multiple steps in food production where unintended allergen presence may occur. While a number of variables were shown to be predictable, unpredictable aspects still remain due to the diversity of factory settings and employee behaviour. Nonetheless, the results of this study and resulting guidance provide awareness and specific risk management options for allergenic particles that can be locally implemented and will benefit all food allergy stakeholders.

Phylogenetic analysis for discovery of potential new allergens from cassava, mango, papaya and pineapple

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Food allergy is an adverse effect that occurs reproducibly on exposure to a particular food due to a specific immune response triggered by an allergen. Most of the reactions are mediated by IgE antibodies and some groups of protein families present in food are responsible for the majority of cases of allergy. However, novel allergens are also emerging as allergy to different food is growing worldwide, especially in Western countries. In addition, similarities among proteins of different species at sequence or structure level may lead to the cross-reactivity, a phenomenon that happens when the antibody recognises an allergen and proteins that share the same epitopes. Thus, there is great interest in the identification of potential food allergens as they may improve the diagnosis of food allergy. In this regard, case reports of allergy to cassava (*Manihot esculenta*) tuberous roots, mango (*Mangifera indica*), papaya (*Carica papaya*) and pineapple (*Ananas comosus*) fruits are growing in Brazil, but the information on their potential allergens is limited. Therefore, we aimed to identify putative novel allergens of these foods by phylogenetic analysis of the allergens already reported in edible related organisms. To achieve this goal, phylogenetic trees for cassava, mango, papaya and pineapple were prepared using the NCBI phyloT tool, and the edible closely related organisms were screened for reported allergens in the Allergome Database. Thereafter, the allergen sequences found in the related organisms were used to BLAST-search for similar proteins in the four foods under study. The cassava genome was searched for sequences similar to allergens reported in *Malpighiaceae* and *Passifloraceae* members of the *Malpighiales* order, which revealed putative cassava allergens belonging to profilins and pathogenesis-related proteins. The phylogenetic proximity to the members of the *Poaceae* family allowed the identification of pineapple potential allergens similar to cupins, prolamins and polcalcins, among others. In the case of mango, protein sequences similar to cupins and prolamins from *Anacardiaceae*, *Rutaceae* and *Sapindaceae* members of the *Sapindales* order were identified as putative allergens. Finally, expansin and enzymes were the potential allergens of papaya, as deduced from the comparison to other members of the *Brassicales* order. These selected sequences from cassava, mango, papaya and pineapple will be cloned and expressed in *Pichia pastoris* and the recombinant proteins will be tested with sera from patients allergic to the four foods under study.

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P14

A simple evaluation of reference materials and analytical methods for the identification of food allergens as a crucial initial step in a validation process

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To protect sensitive individuals from the risk of allergic reactions, allergens have to be declared on the food labels. To ensure compliance with labelling, reliable analytical results are necessary. There are two fundamental parameters of quality assurance: reference materials and analytical methods for the detection of allergens in food products. Reference materials must be sufficiently homogenous, stable and traceable. Due to insufficient availability of certified reference materials, preparation of homemade internal standards is often required. On the other hand, the selection of a commercial kit as an initial phase in the validation of a method is a key point. Enzyme-linked immunosorbent assay (ELISA) is considered as the most widely used technique amongst analytical approaches for the detection of allergens, but its performance might be sometimes compromised. In order to define the capability of a technical procedure to identify and quantify the potency of allergenic extracts and be able to develop validated methods, an initial and simple approach based on spiking experiments for the evaluation of possible standards and analytical methods together, must be addressed. Internal standards can be prepared from raw or processed material. In the case of nuts for example, the quantification (mg/kg) of the allergen may vary or not between pastes from raw or toasted material. An initial screening study trying several standards with different commercial ELISA test kits allows to choose the most suitable combination 'material for the preparation of a standard – test kit' according to the specificity of antibodies. Secondly, the approach of a panel of different matrixes to spike with an appropriate amount of a standard provides information about different factors that may influence in the test results: presence of compounds such as polyphenols, reduced solubility of denatured proteins, etc. So, from complex spiking matrixes such as vinegar, orange juice, plum jam, chocolate and hot sauce; limitations of an ELISA test kit due to the acidic pH or the presence of tannins in the sample can be identified. By and large, it provides information about the stability epitopes and the overall performance of the reference material and the analytical method together. In conclusion, the approach described must be considered an essential simple start point in the validation of ELISA-based methods in order to ensure reliability in the detection and quantification of food allergens.

Is there a relation between legume protein consumption and the prevalence of legume sensitisation?**Mark Smits**^{1,2,3}, T.-M. Le^{1,3}, P. Welsing¹, G. Houben^{1,2,3}, A. Knulst^{1,3} and K. Verhoeckx^{1,2}

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Legumes vary considerably in their sensitising potential and allergic response. The reason behind this variation is not fully understood yet. Possible explanations for the variation could be differences in consumption, exposure, food processing and geographical background. The aim of this study was to investigate if there is a correlation between legume protein consumption and the prevalence of legume sensitisation. Furthermore, the relationship between sensitisation and specific peanut allergens and their concentration in peanut was investigated. Sensitisation data from peanut, soybean, lupine, lentil, and pea in the general population were gathered from scientific publications and were analysed in relationship to consumption data obtained from national food consumption survey databases. Data were stratified for children <4 years, children 4-18 years, and adults (>18 years). Additionally, the percentage of legume consumers was investigated. Furthermore, the relative concentration of peanut allergens (Ara h 1, 2, 3, 6, 7, and 8) were compared to specific IgE sensitisation data for these peanut allergens. Correlation was analysed using weighted least squares regression analysis and expressed as a *r* value. Sufficient data on prevalence and consumption was available for peanut (*n*=61) and soybean (*n*=17). Statistical analysis for lupine, lentil, and pea could not be performed. Analysis of all age groups together resulted in a low correlation between peanut sensitisation and the relative consumption (*r*=0.407), absolute consumption (*r*=0.468) and percentage of consumers (*r*=0.243). For soybean, no significant correlation was found between the prevalence of soybean sensitisation and the relative consumption (*r*=0.352), absolute consumption (*r*=0.217) and the percentage of consumers (*r*=0.007). No correlation was found between the relative concentrations of Ara h 1, 2, 3, 6, 7, and 8 and sensitisation to these peanut allergens. Sensitisation to Ara h 2 (70.7%) and Ara h 6 (71.2%) was high, while the concentration of these allergens is low (6.2% and 5.8%, respectively). In contrast, the concentration of Ara h 3 (70.6%) was high while sensitisation was low (37.3%). In conclusion, the results indicate that the amount of consumption plays a minor role, if any, in the prevalence of sensitisation to legume proteins. Furthermore, the concentration of specific peanut allergens in peanut did not correlate with the frequency of sensitisation to these allergens. The prevalence of peanut sensitisation is most likely influenced by other factors such as processing, matrix, frequency, timing and route of exposure, and patient factors.

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Microsphere-based multiplex technology for the simultaneous detection of food allergens

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The obligation to provide customers with allergy information according to Regulation (EU) No 1169/2011 is an enormous challenge. This is especially true for restaurants and catering services, because these companies now need to address the presence of all 14 allergens in their prepared food and thus need full knowledge on their ingredients, and at the same time also need to control and avoid cross contamination during preparation and processing of their served foods. In order to test their ingredients and avoid allergic reactions in sensitive consumers, these companies need some simple tests. Most laboratory allergen tests are based on the ELISA detection format and, for on-site measurement, some lateral flow devices (LFDs) are available. However, both format types are focussed on detecting one allergen, e.g., an LFD for detecting peanut. Detecting all 14 allergens in a laboratory is already an analytical challenge by itself, detecting these 14 with a simple on-site test is another one. The Luminex xMAP technology offers new possibilities as it enables the detection of multiple targets in a customised way. The technology is based on distinct colour encoded microspheres. For the purpose of allergen detection, we decided to use the MAGPIX, as it can detect up to 50 parameters at the same time and is also robust and transportable, thus enabling multiple on-site measurements. A small pilot study was performed for 4 allergens: cashew, almond, hazelnut and lupine. Detection of the three tree nut allergens was based on specific monoclonal antibodies and that of the legume lupine, was based on a specific polyclonal antibody. Binding of allergens was envisioned in the so-called immuno sandwich model. Although no optimisation was performed yet, the first preliminary results with pure ingredients (allergens) already indicated high sensitivity for detecting the 4 allergens and the 4 assays could easily be combined into one multiplex assay. In addition, several real samples were prepared, i.e., 'healthy gluten and sugar-free bars', cookies and a chocolate sample, by a simple straight forward extraction with PBS buffer and subsequently tested in the newly developed MAGPIX 4-plex method. In conclusion, the MAGPIX offers a feasible way for the custom-made multiple on-site detection of allergens in food.

Production of well characterised incurred chocolate bars for the development of a harmonised quantitative MS reference method

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One of the aims of the EFSA Thrall project is to develop a harmonised quantitative mass spectrometric reference method for the detection of allergens in food. For this it is important to have well characterised incurred test material to include the effect of food processing on the detectability and quantification of the food allergens. One of the test matrices chosen for the ThRAIL project is a chocolate bar. Confectionary products, to which chocolate bars belong, are responsible for 9% of the food recalls based on allergens, resulting in a third place following baked products and ready to eat meals. Furthermore, they are a challenging matrix for allergen analysis because of their high fat and polyphenol content. The target food allergens selected were cow's milk, soybean, tree nuts (hazelnut, almond), hen's egg, and peanut since all are on Annex II of the EU-FIR, cause severe IgE-mediated reactions, are responsible for recalls and pose issues in PAL labelling. A well characterised incurred test material requires well characterised food allergens to be included. The ThRAIL approach has been to start from commercially available qualified materials. Whenever these are not available or economically not realistic newly sourced materials have to be qualified in a manner similar to reference materials with regards to total protein content, protein profile, allergen content and allergen activity. Chocolate bars with allergenic ingredients incurred at different concentrations (40, 10, 4, 2 and 0 mg total allergen protein/kg commodity) were produced through serial dilution. Homogeneity was assessed with ELISA according to Fearn and Thompson [1]. This poster will describe the entire process starting from the characterisation of the food allergens, through the production of the chocolate bars and the homogeneity testing.

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Development of a model for predicting allergenicity of new and modified proteins

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Current Codex Alimentarius (2009) and EFSA guidance indicates that sequence identity to known allergens is acceptable for predicting the cross-reactive potential of novel proteins and resistance to pepsin digestion and glycosylation status for evaluating allergenicity potential de novo. Other physicochemical and biochemical protein properties, however are not used in the current weight of evidence approach. In this study, we have used the Random Forest Method for developing an *in silico* model that yields a prediction of the allergenic potential of a novel protein based on its shared physicochemical and biochemical properties with known allergens. The protein data used in this study were obtained from the SwissProt database. Regarding the allergenicity of the proteins, we have used the information data from the COMprehensive Protein Allergen REsource (COMPARE) database. In our analyses, we considered all proteins in the COMPARE database as allergenic. Initially, a pilot study was performed to determine the appropriate amount and types of data. In total 138 variables were tested (such as biochemical (e.g., biological function) and physicochemical properties (e.g., molecular weight), sequence-based features (e.g., number of specific amino acids) and subcellular locations (e.g., cytoplasm or membrane). In addition, we have used variables which were calculated based on the amino acid sequence by means of the ProtParam software, the EpiTOP tool (predicting epitopes), the PSIPred Protein Sequence Analysis program and DiANNA (prediction S_S bridges). Based on the pilot experiments we excluded qualitative descriptors; for example, keywords and go-annotations were excluded due to uncertainty of the conscientiousness with which they were recorded and large amount of missing values; other considerations were made to reduce the variable set to those that were most complete and objective. In the final model, we used 29 variables calculated from the protein sequence. Initially, results showed a good model performance with a sensitivity, specificity and accuracy of 89%. This was validated with three different sets: animal, fungi and plant, and a set containing all kingdoms. Furthermore, we tested the model with a completely new data set. All tests showed accuracy, specificity and sensitivity of 85% or more. The applicability of the validated Random Forest model was successfully tested using mealworm proteins. Since the model only requires the protein sequence for calculations it can be easily implemented into the existing allergenic risk assessment approach.

Milk allergen detection in food products by DNA-based methods: exploiting mitochondrial and nuclear gene markers from cow

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Cow's milk allergy is one of the most common food allergies in early childhood, which can persist through adult life. The current effective treatment for milk allergy is the adoption of an elimination diet. However, accidental exposure to cow's milk proteins is recurrent since they are present in uncounted food products, as ingredients (cheeses, yogurts) or as technological aids (sausages, cooked hams), exposing allergic individuals to a constant threat, even with the ingestion of trace amounts [1]. The aim of this work was to screen different genes of cow genome in order to develop a specific, sensitive and accurate method to detect trace quantities of milk in complex and processed foods. Model mixtures containing known quantities (10% to 0.0001%, n=13) of cow's milk protein concentrate (technological aids) in turkey meat were prepared and submitted to two processing methods, simulating the production of cooked ham (67°C, 5 h) and sausages (121°C, 15 min). DNA extraction was performed using the NucleoSpin Food kit (Macherey-Nagel, Düren, Germany). Sequences of mitochondrial (12S rRNA, cyt b, among others) and nuclear (β -lactoglobulin and caseins) genes from cow and other relevant animal species were retrieved from NCBI database. Specific primers and TaqMan probes were designed and tested in model mixtures by qualitative PCR and real-time PCR. Most of the developed PCR assays presented absolute limits of detection (LOD) between 10 and 1 pg of cow's DNA and relative LOD of 0.005% of cow's milk protein concentrate in raw meat mixtures and respective autoclaved sausages and cooked hams. The specificity study with some mitochondrial genes revealed cross-reactivity with meat species, including turkey, pork, goat and chicken; while using nuclear genes the method was specific to cow. Moreover, the use of TaqMan probes seems to improve the PCR efficiency and linearity of the method. From the obtained results, real-time PCR targeting the 12S rRNA gene with a TaqMan probe showed adequate performance parameters (PCR efficiency=108.8%, $r^2=0.980$, slope=-3.127) in the range 10-0.005% of cow milk protein concentrate in processed ham. Currently, more tests are being done by real-time PCR with specific probes in order to improve the specificity and the sensitivity of the method for milk detection in food products.

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