3RD INTERNATIONAL CONFERENCE

# FOOD ALLERGY FORUM

TOWARDS A FOOD ALLERGY-FREE WORLD

27-29 SEPTEMBER 2023 AMSTERDAM

ABSTRACTS OF LECTURES & POSTERS

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IN COLLABORATION WITH TNO CONTROLLING THE IMMUNE EXPOSOME PROGRAMME

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## CONTENTS



Key to the abstracts of lectures and posters:

- 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

## WELCOME

The main objectives of the **FOOD ALLERGY FORUM** are: providing a unique platform for the food industry, science, and regulatory authorities 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 3rd international conference of the **FOOD ALLERGY FORUM** is organised in collaboration with the TNO Controlling the Immune Exposome programme. The conference topics are intended to meet the needs of all stakeholders in the food chain, food researchers, food and healthcare professionals, focusing on:

- Food allergy development
  What do we currently know about the role of environmental factors, the microbiome, and resulting immune effects?
- Food allergen management
  - How can we assure safe food choices? To answer this question, we need to know where the risks are, what influences food choices, and what we actually eat!
  - How can we verify food safety? Once we know what we eat, we need to know what's in it!
- Food allergy management and therapy
  Characterising and curing the patient, where are we with that?

In addition, the conference includes:

- ILSI Europe training session 'Demystifying the risks on allergy risk assessment'
- SCIEX workshop 'LC-MS/MS for qualitative and confirmatory analysis of allergens'.

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

| | |
|---|---|
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| Prof. Harry Wichers | Wageningen University & Research, the Netherlands |

## PROGRAMME AT A GLANCE

### WEDNESDAY 27 SEPTEMBER 2023

| | | |
|---|---|---|
| 09:30 – 12:30 | ILSI Europe Training Session<br>*Demystifying the risks on allergy risk assessment* | |
| 13:00 – 13:15 | **FOOD ALLERGY FORUM**<br>*Opening of the 3rd international conference* | Exhibition |
| 13:15 – 14:15 | Opening session<br>*Towards a food allergy-free world: Looking back and forward* | |
| 14:15 – 15:15 | Session 1<br>*Aspects of food allergy development* | |
| 15:15 – 15:45 | Networking break & poster viewing | |
| 15:45 – 17:05 | Session 1 (contined) | |
| 17:05 – 17:35 | Company pitches<br>*Short presentation by sponsors/exhibitors* | |
| 17:35 – 18:00 | Speed presentations<br>*Short presentation by selected poster presenters* | |
| 18:00 – 19:00 | Poster viewing & informal get-together | |

### THURSDAY 28 SEPTEMBER 2023

| | | |
|---|---|---|
| 08:30 – 10:15 | Session 2<br>*Food allergen management: How can we assure safe food choices?* | Exhibition |
| 10:15 – 10:45 | Networking break & poster viewing | |
| 10:45 – 12:45 | Session 3<br>*Food allergen management: How can we verify food safety?* | |
| 12:45 – 14:15 | Lunch break<br>Workshop presented by SCIEX<br>*LC-MS/MS for qualitative and confirmatory analysis of allergens* | |
| 14:15 – 15:45 | Session 4<br>*Food allergy management and therapy* | |
| 15:45 – 16:15 | Networking break & poster viewing | |
| 16:15 – 17:15 | Session 4 (continued) | |

### FRIDAY 29 SEPTEMBER 2023

| | | |
|---|---|---|
| 08:45 – 10:15 | Session 5<br>*ILSI Europe – Food Allergy Task Force:*<br>*25 years developing allergen risk assessment and management* | Exhibition |
| 10:15 – 10:45 | Networking break & poster viewing | |
| 10:45 – 12:45 | Final session<br>*Towards a food allergy-free world: Looking back and forward* | |
| 12:45 | **FOOD ALLERGY FORUM**<br>*Closing of the 3rd international conference* | |

**WEDNESDAY 27 SEPTEMBER 2023**

**ILSI EUROPE TRAINING SESSION**

**DEMYSTIFYING THE RISKS ON ALLERGY RISK ASSESSMENT**

09:30 – 12:30

During this training, ILSI Europe experts will clarify the methodologies of allergen quantitative risk assessment, ultimately improving decision-making on precautionary labelling. Participants will gain valuable insights into the potential risks posed to allergic consumers by unexpected allergen presence in food products and learn how to effectively mitigate these risks.

As a participant in this training, you will have the unique opportunity to join our Community of Practice. This platform allows you to stay informed on the latest updates on quantitative risk assessment and share your concerns with the experts on the topic.

Presenters:

- Dr Simon Flanagan, Head of Speciality Analysis and Food Allergen Services at Mondelēz International
- Prof. René Crevel, Director at René Crevel Consulting
- Dr Neil Buck, Global Toxicology and Ecotoxicology Expert at General Mills
- Dr Marty Blom, TNO, the Netherlands

Programme:

09:30 Introduction to ILSI and Guidance and Code of Practice (CoP)
09:45 Core principles
10:05 Communication across supply chains
10:25 Management of operations
10:45 Q&A
11:00 Coffee break
11:15 Management of incidents
11:35 Guiding Principles: politics, calculation of reference doses
11:55 Guiding Principles: consumption, form of contaminations
12:15 Q&A

**WEDNESDAY 27 SEPTEMBER 2023**

13:00 Opening of the **FOOD ALLERGY FORUM** – 3rd international conference
Prof. Geert Houben – conference chair

**OPENING SESSION**
**TOWARDS A FOOD ALLERGY-FREE WORLD: LOOKING BACK AND FORWARD**

*In 2019, on the last day of the 2nd international conference of the **Food Allergy Forum**, the following questions were addressed: Where are we in 2025 in (i) protecting existing food allergy sufferers, (ii) curing food allergy, and (iii) preventing food allergy. At the start of this conference, we will pick up where they left off and elaborate on: "How far are we now on the road to a food allergy-free world?"*

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

13:15 *Protecting existing food allergy sufferers: where are we now?*
Prof. Samuel Godefroy, Food Science Department, Laval University, Canada

13:35 *Curing food allergy, where are we now?*
Prof. André Knulst, Department Dermatology/Allergology, UMC Utrecht, the Netherlands

13:55 *Preventing food allergy: where are we now?*
Prof. Gideon Lack, Department of Women and Children's Health, King's College London, UK

**SESSION 1**
**ASPECTS OF FOOD ALLERGY DEVELOPMENT**

*During the past decade, our knowledge regarding factors that play a role in the development of food allergy has increased. What do we currently know about the role of environmental factors, the microbiome, and resulting immune effects?*

Chair: Prof. Johan Garssen
Danone Nutricia Research and Utrecht University, the Netherlands

14:15 *Chair's introduction*

14:20 *Food allergen bio-corona on microplastics*
Dr Dragana Stanić-Vučinić, Center of Excellence for Molecular Food Sciences, University of Belgrade, Serbia

14:40 *Microbes, mucus, and food allergy*
Dr Mahesh Desai, Department of Infection and Immunity, Luxembourg Institute of Health, Luxembourg

15:00 *Gut microbiome -omics in the field of food allergies*
Dr Ana G. Abril, Department of Microbiology and Parasitology, University of Santiago de Compostela and Department of Food Technology, IIM-CSIC, Spain

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

**WEDNESDAY 27 SEPTEMBER 2023**

**SESSION 1 (continued)**

15:45 *Edible insects as a novel source of food allergens*
Dr Isabel Mafra, REQUIMTE-LAQV, University of Porto, Portugal

16:05 *The role of skin barrier in developing IgE-mediated food allergy*
Dr Marloes van Splunter-Berg, Cell Biology and Immunology Group, Wageningen University & Research, the Netherlands

16:25 *Allergen-specific B cell responses in food allergy*
Dr Willem van de Veen, Swiss Institute of Allergy and Asthma Research, Switzerland

16:45 *Could the real Treg please stand up? The hunt for the elusive peanut allergy-suppressing T helper subset*
Dr Victor Turcanu, Department of Women and Children's Health, King's College London, UK

**COMPANY PITCHES & SPEED PRESENTATIONS**

Chair: Dr Marty Blom, TNO, the Netherlands

17:05 **Company pitches**
Short presentations (5-minutes) by sponsors/exhibitors to inspire the audience to visit their booths
*Gold Standard Diagnostics – Romer Labs – Sciex – Inbio – Nutrilab – R-Biopharm*

17:35 **Speed presentations**
Selected poster presenters are given 5 minutes to present an overview of their research

P1 *Long-term tolerance to cashew nut after low dose oral immunotherapy in preschool-aged children*
Lieke Barten, Paediatric Allergy Treatment Centre, Deventer Hospital and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, the Netherlands

P5 *Neglected wheat species as a source of hypoallergenic lines: Unlocking the potential through genetic exploration*
Dr Lisa Call, Department of Crop Sciences, University of Natural Resources and Life Sciences, Austria

P17 *Molecular dynamics simulation of allergens in food: An in silico approach*
Dr Amin Mousavi Khaneghah, Department of Fruit and Vegetable Product Technology, Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology, State Research Institute, Poland

P21 *Developing an ip animal model to predict sensitising capacity of novel food*
Behnaz Shafie, National Food Institute, Technical University of Denmark, Denmark

18:00 – 19:00
**Poster viewing & informal get-together**

19:00 **END OF DAY 1**

**THURSDAY 28 SEPTEMBER 2023**

**SESSION 2**
**FOOD ALLERGEN MANAGEMENT**

*How can we assure safe food choices? To answer this question, we need to know where the risks are, what influences food choices, and what we actually eat!*

Chair: Lotte Neergaard Jacobsen
Arla Food Ingredients, Denmark

08:30 *Chair's introduction*

08:35 *Expect the unexpected – food trends and challenges in allergen management*
Ronald Niemeijer, R-Biopharm, Germany

08:55 *Global patterns in food-induced anaphylaxis*
Dr Paul Turner, National Heart & Lung Institute, Imperial College London, UK

09:15 *Accidental food-allergic reactions: are people taking risks or simply misunderstanding allergen information?*
Dr Rebecca Knibb, Applied Health Research Group, Aston University, UK

09:35 *To eat or not to eat: Adherence to dietary advice after food challenges*
Dr Thuy-My Le, Department Dermatology/Allergology, UMC Utrecht, the Netherlands

09:55 *How much we eat – food intake data in food allergen risk assessment*
Marie Meima, M.Sc., TNO, the Netherlands

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

**THURSDAY 28 SEPTEMBER 2023**

**SESSION 3**
**FOOD ALLERGEN MANAGEMENT**

*How can we verify food safety? Once we know what we eat, we need to know what's in it!*

Chair: Dr Bert Popping
FOCOS, Germany

10:45 *Chair's introduction*

10:50 *AOAC Food Allergens Working Group: Development of guidance for method developers and end users*
Dr Melanie Downs, Food Science and Technology Department, University of Nebraska-Lincoln, USA

11:10 *Standardization of a reference prototype-based method to quantify food allergens in complex foods and compliance with reference doses: an outcome of the ThRAll project*
Dr Linda Monaci, Institute of Sciences of Food Production (ISPA-CNR), Italy

11:30 *Reliable immunosensing platforms for the multiplexed determination of major allergens*
Dr Sergi Morais, Department of Chemistry, Polytechnic University of Valencia, Spain

11:45 *Food allergen detection, a complete and versatile solution covering all rapid testing methods*
Cristina Romero, Gold Standard Diagnostics, Spain

12:00 *Expanding the scope of a routine LC-MS/MS approach to allergens testing*
Dr Jianru Stahl-Zeng, Sciex, Germany / Daniel McMillan, Sciex, UK

**SUBMITTED ABSTRACTS**

12:10 *Peptides, proteins, and conversion factors: how do they affect my measurement uncertainty?*
Jørgen Vinther Nørgaard, European Commission, Joint Research Center, Belgium

12:20 *Multi-allergen quantification in food using concatemer-based isotope dilution mass spectrometry: A collaborative study*
Dr Maxime Gavage, CER Groupe, Belgium

12:30 *Comparative study of egg and celery allergen ELISA and DNA kits in different food matrices*
Dr Nathalie Smits, Wageningen Food Safety Research, the Netherlands

12:45 **Lunch break**

13:00 – 14:00
*Workshop LC-MS/MS for qualitative and confirmatory analysis of allergens*
(for details, see page 9)

**THURSDAY 28 SEPTEMBER 2023**

**WORKSHOP SPONSORED AND PRESENTED BY SCIEX**


**LC-MS/MS FOR QUALITATIVE AND CONFIRMATORY ANALYSIS OF ALLERGENS**

13:00 – 14:00

Food allergies are the leading cause of anaphylaxis, an acute, potentially deadly allergic reaction. The prevalence and severity of food allergies are rising, with approximately 150 million people suffering from food allergies worldwide. Presently, there is no cure for food allergies, and sufferers must rely on the correct labelling of foods to avoid consuming allergens. Hence, developing sensitive and accurate analytical methods to screen for the presence of allergens in food products is necessary to prevent potentially life-threatening health problems for allergy sufferers.

The SCIEX vMethod applications for food allergen testing previously provided a workflow for sample preparation and LC-MS/MS detection of 12 distinct allergens, including egg, milk, almond, Brazil nut, cashew, hazelnut, pine nut, pistachio, pecan, walnut, peanut and soy, and for the quantification of gluten.

In 2021, the Food Allergy Safety, Treatment, Education, and Research (FASTER) Act was passed in the United States. The FASTER Act requires all foods sold in the United States that contain sesame to declare it as an ingredient or state “Contains: Sesame” immediately after the ingredient list. After evaluating 24 different sesame peptides, 2 of the most sensitive peptides were selected and added to the SCIEX vMethod.

In this workshop, applications experts from SCIEX will discuss the challenges of developing and expanding the LC-MS/MS workflow and verifying it to the standards required to achieve its First Action Official Method (FAOM) classification from AOAC International.

**THURSDAY 28 SEPTEMBER 2023**

**SESSION 4**
**FOOD ALLERGY MANAGEMENT AND THERAPY**

*Characterising and curing the patient, where are we with that? And in the end… what is the food allergic patient's perspective on food allergen and allergy management?*

Chair: Todd D. Green, MD
DBV Technologies, USA

14:15 *Chair's introduction*

14:20 *Core Outcome Set for IgE mediated food allergy: What to measure?*
Dr Daniel Munblit, Division of Care for Long Term Conditions, King's College London, UK

14:40 *Immune signatures during oral food challenges*
Dr Annette Kuehn, Department of Infection and Immunity, Luxembourg Institute of Health, Luxembourg

15:00 *HMOs and immunomodulation: potential for the management of allergic disorders?*
Prof. Johan Garssen, Utrecht Institute for Pharmaceutical Sciences, Utrecht University and Nutricia Research, the Netherlands

15:20 *Good food does exist: impact of specific food components (dietary fibre and omega-3 PUFAs) on allergy*
Prof. Harry J. Wichers, Wageningen Food and Biobased Research, Wageningen University & Research, the Netherlands

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

16:15 *The potential role for epigenetics in the management and treatment of food allergy*
Dr Jörg Tost, Laboratory for Epigenetics & Environment, Centre National de Recherche en Génomique Humaine, CEA-Institut de Biologie François Jacob, France

16:35 *A new perspective on oral immunotherapy for food allergy, age matters?*
Dr Ted Klok, Paediatric Allergy Treatment Centre, Deventer Hospital, the Netherlands

16:55 *Update on sublingual immunotherapy for peanut allergy*
Dr Edwin Kim, Department of Pediatrics, UNC School of Medicine, USA

17:15 **END OF DAY 2**

**FRIDAY 29 SEPTEMBER 2023**

**SESSION 5**
**ILSI EUROPE – FOOD ALLERGY TASK FORCE:**
**25 YEARS DEVELOPING ALLERGEN RISK ASSESSMENT AND MANAGEMENT**

*For more than two decades, the Food Allergy Task Force has followed an ambitious aim: to establish consensus among multiple stakeholders on science-based approaches for food allergen risk assessment and management. In this session, an overview of our most recent major projects as well as a roadmap of our new and future activities will be presented.*

Chair: Prof. René Crevel, René Crevel Consulting, UK

08:45 *ILSI Europe Food Allergy Task Force: a multistakeholder approach to address the risks in food allergen management to protect all consumers*
Prof. René Crevel, René Crevel Consulting, UK

08:55 *Allergenicity assessment of new protein-containing sources and ingredients*
Prof. René Crevel, René Crevel Consulting, UK

09:15 *Food allergen quantitative risk assessment (QRA): a multistakeholder approach to improve consisency in the application of QRA by food business operators*
Dr Benjamin Remington, Remington Consulting Group BV, the Netherlands

09:35 *How do we effectively manage allergens and communicate risk of products with PAL to the consumer with food allergies?*
Dr Simon Flanagan, Mondelēz International, UK

09:55 Interactive Q&A

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

**FRIDAY 29 SEPTEMBER 2023**

## FINAL PLENARY SESSION
## TOWARDS A FOOD ALLERGY-FREE WORLD: LOOKING BACK AND FORWARD

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

10:45 *Chair's introduction*

10:50 *FAO/WHO Expert Consultation on Risk Assessment of Food Allergens*
Dr Kang Zhou, Food Safety and Quality Unit, Food and Agriculture Organization of the United Nations (FAO), Italy

11:15 *PAL-ing around with allergens: analytical conundrums and legal predicaments – a dialogue*
The FAO and WHO have released the expert view on food allergen reference doses and precautionary allergen labelling. The challenges faced by the food industry in regard to analytics and labelling will be discussed from the perspectives of a food analyst, a food lawyer, and a food manufacturer.

- Dr Bert Popping, FOCOS, Germany
- Cesare Varallo, Food Law Latest, Italy
- Antje Preußker, Lebensmittelverband Deutschland, Germany

12:15 *Allergenicity assessment of novel foods, the way forward*
Dr Kitty Verhoeckx, Department Dermatology/Allergology, UMC Utrecht, the Netherlands

12:35 *Lessons learned on our journey towards a food allergy-free world*
Prof. Geert Houben, TNO and University Medical Center Utrecht, the Netherlands

12:45 Closing of the **FOOD ALLERGY FORUM** – 3rd international conference

*Take your packed lunch to eat along the way!*

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# LECTURE ABSTRACTS

## OPENING SESSION
## TOWARDS A FOOD ALLERGY-FEE WORLD: LOOKING BACK AND FORWARD

*In 2019, on the last day of the 2nd international conference of the Food Allergy Forum, the following questions were addressed: Where are we in 2025 in (i) protecting existing food allergy sufferers, (ii) curing food allergy, and (iii) preventing food allergy? At the start of this conference, we will pick up where they left off and elaborate on: "How far are we now on the road to a food allergy-free world?"*

### PROTECTING EXISTING FOOD ALLERGY SUFFERERS: WHERE ARE WE NOW?

**Samuel Godefroy**

Food Risk Analysis and Regulatory Excellence Platform (PARERA), Food Science Department and Institute of Nutrition and Functional Food (INAF), Laval University, Canada

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At the last gathering of the Food Allergy Forum (2nd international conference, 1-3 April 2019), we reviewed progress being made to equip food allergy sufferers and their care takers with the tools they need to enhance the prevention of food allergy incidents. At the time, domestic and international jurisdictions were progressing in updating guidance on food allergen management, development of scientific evidence to support updated determination of priority allergens or risk assessment approaches. We all contemplated the development of initiatives aiming to improve the quality of life of food allergic consumers and their families: from smart food labels to the development of certification protocols for food allergen management practices, enhanced and more integrated measures seemed feasible.

Almost 4 years later, this presentation will review what was accomplished to date and where more effort seems to be warranted in the upcoming period. Even though it is aspirational, we are still aiming to progress significantly towards a food allergy incident free environment by 2025.

## CURING FOOD ALLERGY – WHERE ARE WE NOW?

**André Knulst**

Department Dermatology/Allergology, UMC Utrecht, the Netherlands

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Curative treatments for food allergy were and are widely studied, and slow progress is made. Thus far, only one treatment has been approved by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA): oral immunotherapy for peanut allergy in children 4-17 years of age. Reimbursement is still pending. So, the majority of patients still have to adhere to avoidance diets. The main routes for immunotherapy that are investigated so far are the oral and epicutaneous route, but also the sublingual, intralymphatic, and rectal route were explored. Many other options were explored of which biologics and newer drugs that are becoming available seem the most promising.

*Immunotherapy: the current status*
Studies are underway for peanut, cow's milk, hen's egg, and nuts, mostly focusing on oral immunotherapy (OIT). Current issues are the limited effectiveness. The threshold is raised, but complete tolerance not always achieved. There are frequent and sometimes severe side effects. It is unclear whether there is a long-term effect or not. Recently, epicutaneous immunotherapy (EPIT) for peanut appeared successful in toddlers (1-3 years). Studies in older children are underway. Side effects in EPIT are compared to OIT, but effectiveness might be lower. There are early studies investigating sublingual IT (SLIT) also using (peanut) peptides.

*Biologics: promising novel drugs?*
For other atopic diseases as eczema and asthma, biologics appeared very effective. For food allergy omalizumab (anti-IgE), an effective drug in asthma has been studied for food allergy. Omalizumab was able to enhance the threshold for peanut and other foods, to diminish side effects during OIT for peanut, and was effective in combination with OIT. Treatment with dupilumab (anti-IL4/13 receptor), which was very effective in atopic eczema, appeared to decrease IgE levels up to 80% over 3 yrs. Whether this results in clinical improvement is still unknown. A novel anti-IgE (ligelizumab), with much higher affinity to the high affinity IgE receptor is currently studied in peanut allergy and multiple food allergy. Given the broader effect (not only for specific foods), these drugs may have high potential.

*Other drugs*
Many more drugs were and are being explored, including pre- and probiotics, other biologics, BTK and JAK inhibitors, and mast cell inhibitors. The future will learn which are the most promising and whether combination therapy might be an even better option.

## PREVENTING FOOD ALLERGY: WHERE ARE WE NOW?

**Gideon Lack**

Department of Women and Children's Health, King's College London, UK

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The prevalence of food allergies has increased in many countries with a noticeable rise in peanut allergy in the UK, North America, and Australia. IgE mediated food allergies in the UK and North America are currently estimated at 8% in school-age children. The prevalence of peanut allergy in the UK, North America, and Scandinavian countries is about 2% in schoolchildren. The prevalence of tree nut allergies is approximately 1% in the UK and North America. These allergies are rarely outgrown and persist into adulthood.

Guidelines in the UK and North America in the early 2000s recommended avoidance of peanuts (and in the US other food allergens) during pregnancy, breastfeeding, and the first year of the infant's life, based on the belief that avoidance of allergenic foods in infancy will prevent peanut and other food allergies from occurring (despite no evidence). In the same period, there has been emphasis on WHO guidelines advocating exclusive breastfeeding during the first six months of life. During the first decade of the millennium, there has been an increase in peanut allergy. In contrast, since 2015, there has been a growing body of evidence that early introduction of peanut into the infant's diet at 4-6 months of age will protect against the development of peanut allergy, and high evidence that early introduction of egg at 4-6 months of age will prevent the development of egg allergy. There is low level evidence cow's milk allergy can be prevented with early introduction of cow's milk [1]. The LEAP (Learning Early about Peanut Allergy) study showed that early introduction of peanut during infancy in high-risk babies with severe eczema or egg allergy could prevent 81% of cases of peanut allergy [2]. This protection continued even when the children who originally consumed peanuts completely avoided peanut between the ages of 5-6 years of life [3]. A Scandinavian study of several thousand normal infants who were at low risk of peanut allergy reproduced the results of the LEAP study showing 60-75% reduction in peanut allergy if peanut butter was introduced between 3-6 months of life and eaten at least three times per week [4].

Despite guidelines now advocating early introduction of peanut, egg, and other allergy-causing foods in the first year of life, they appear to have had limited impact. In 2009, Australian infants were largely avoiding peanuts and the rate of peanut allergy was 3.1% [5]. In 2019, after the change in guidelines in 2015, about 90% of Australian infants were eating peanut in the first year of life but peanut allergy was still elevated at 2.6% of the population [6]. The main obstacles to effective prevention of peanut and other food allergies are the delayed introduction of these foods into infants' diets and insufficient quantities of these foods consumed. An integrated meta-analysis of the LEAP and EAT study cohorts [7] shows that introduction of peanut should ideally occur in high-risk infants with eczema and non-Caucasian ethnicities by four months of age, and in lower risk infants by six months of age. The efficacy of preventing peanut allergy decreases significantly over time if peanuts are introduced after six months. Weaning guidelines should be updated to encourage infants to eat at least the equivalent of two teaspoons of peanut butter per week and the equivalent of one whole egg per week by six months of age, and in higher-risk infants by four months of age. Such guidelines together with advice and support from healthcare professionals will considerably reduce the burden of food allergies.

### References

1. Scarpone, R. *et al.*, 2023. JAMA Pediatrics 177: 489-497.
2. Du Toit, G. *et al.*, 2015. The New England Journal of Medicine 372: 803-813.
3. Du Toit, G. *et al.*, 2016. The New England Journal of Medicine 374: 1435-1443.
4. Ove Skjerven, H. *et al.*, 2022. The Lancet. 399: 2398-2411.
5. Osborne, N.J. *et al.*, 2011. Journal of Allergy and Clinical Immunology 127: 668-76.e1-2.
6. Soriano, V.X. *et al.*, 2022. JAMA 328: 48-56.
7. Roberts, G. *et al.*, 2023. Journal of Allergy and Clinical Immunology 151: 1329-1336.

## SESSION 1
## ASPECTS OF FOOD ALLERGY DEVELOPMENT

*During the past decade, our knowledge regarding factors that play a role in the development of food allergy has increased. What do we currently know about the role of environmental factors, the microbiome, and resulting immune effects?*

### FOOD ALLERGEN BIO-CORONA ON MICROPLASTICS

Tanja Cirkovic Velickovic and **Dragana Stanić-Vučinić**

Faculty of Chemistry, University of Belgrade, Serbia

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Human exposure to nano- and microplastics (NMPs) is a reality, and contamination of food with NMPs has become an issue worldwide. Due to their small size and pervasive nature, NMPs may enter the human body through various routes, ingestion being the most relevant route for food and beverages contaminated with NMPs. The presence of NMPs in the GIT allows them to reach different parts of the body, where they may have both physical and chemical effects.

GIT is also the first point of contact of food allergens and NMPs. To clarify effects of food allergens adsorption and bio-corona formation on NMPs, we have examined binding and structural changes of several allergenic food proteins (ovalbumine, beta-lactoglobuline, tropomyosine) to the polystyrene, polypropylene, and polyethylene terephthalate microplastics. Our results show high binding affinity of food allergens to the plastic particles and structural and functional changes in the allergenic food proteins bound in soft and hard corona. Furthermore, presence of NMPs in the digestive fluid may influence digestion of allergenic food proteins. Our study on the pepsin digestion of milk proteins in the presence of PS showed that cow's milk proteins (caseins) and their larger fragments preferentially bind to the hard corona of PS-MPs during the gastric digestion of milk leading to prolonged survival of larger peptides (particularly of αS2-casein) in the simulated gastric fluid. In future studies, it will be particularly relevant to further study impact of nanoplastics on allergenic proteins digestion and bioavailability. Larger surface area of nanoplastics in comparison to microplastics may provide more anchoring points for proteins and digestive enzymes to tightly bind in the hard corona, causing structural changes and possibly impacting digestive enzymes' function. In the future, particular attention should be given to infant, elderly, and groups already showing digestion impairment, as most of the studies conducted so far consider only healthy adult digestion. However, data so far obtained at the molecular level suggest adsorption and structural changes in allergenic food proteins, particularly in bio-corona around NMPs and impairment of function of digestive enzymes. Studies are warranted on the impact of NMPs/allergen co-exposure on allergenicity and sensitizing potential of allergenic foods contaminated with NMP to fully understand possible impact of NMPs on development and severity of food allergies.

**Acknowledgments**
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**MICROBES, MUCUS, AND FOOD ALLERGY**

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Alterations in the gut microbiome, including diet-driven changes, are linked to the rising prevalence of food allergy, yet little is known about how specific gut bacteria incite breakdown of oral tolerance. Here, we show that depriving specific-pathogen-free mice of dietary fibre leads to a gut microbiota signature containing an increase of the mucin-degrading bacterium *Akkermansia muciniphila*, which is associated with the intestinal barrier dysfunction, increased expression of type 1 and 2 cytokines and IgE-coated commensals in the colon. These changes manifest into exacerbated allergic reactions to food allergens, ovalbumin, and peanut. To demonstrate the causal role of *A. muciniphila*, we employed a tractable synthetic human gut microbiota in gnotobiotic mice. The presence of *A. muciniphila* within the microbiota, combined with fibre deprivation, resulted in stronger anti-commensal IgE coating, and innate type 2 immune responses, which worsened symptoms of food allergy. Our study sheds important light on how gut microbes regulate immune pathways of food allergy in a diet-dependent manner.

## GUT MICROBIOME -OMICS IN THE FIELD OF FOOD ALLERGIES

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Food allergies (FA) have dramatically increased in recent years, particularly in developed countries. It is currently well-established that food tolerance requires the strict maintenance of a specific microbial consortium in the gastrointestinal (GI) tract microbiome as alterations in the gut microbiota can lead to dysbiosis, causing inflammation and pathogenic intestinal conditions that result in the development of FA. Although there is currently not enough knowledge to fully understand how the interactions between gut microbiota, host responses, and the environment cause food allergies [1]. Recent advances in '-omics' technologies (i.e., proteomics, genomics) and in approaches involving systems biology suggest future headways that would finally allow the scientific understanding of the relationship between gut microbiome and FA. This presentation summarizes the insights in the field of FA and the study of GI tract microbiome applying -omic techniques, such as metagenomics and metaproteomics [2,3].

**References**

1. Abril, A.G. *et al.*, 2023. International Journal of Molecular Sciences 24: 2234.
2. Mok, K. *et al.*, 2021. Journal of Fungi 7: 748.
3. Kingkaw, A. *et al.*, 2020. PeerJ 8: e9988.

## EDIBLE INSECTS AS A NOVEL SOURCE OF FOOD ALLERGENS

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In search for sustainable solutions, entomophagy is being proposed as an alternative source of proteins, with economic and environmental advantages when compared to meat. Edible insects are considered a valuable source of nutrients such as polyunsaturated fatty acids, essential amino acids, micronutrients, and proteins, having a nutritional value comparable or superior to those of both chicken and beef. Moreover, edible insects are valuable sources of bioactive compounds after their gastrointestinal digestion that originates small peptides with important bioactive properties [1]. On the other hand, edible insects might induce allergic reactions [2]. Following the guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283 [3], to date, the European Union (EU) has authorised the placing on the market of four species of insects that comply with the legislation on novel foods for human consumption, i.e., *Tenebrio molitor* larva (yellow mealworm), *Locusta migratoria* (migratory locust), *Acheta domesticus* (house cricket) and, recently, *Alphitobius diaperinus* larva (lesser mealworm). The establishment of legislation ensuring the safety of insects for human consumption as food and their availability as edible products, such as frozen, paste, dried and powder forms, are two factors in favour of their general acceptability. The yellow mealworm was the first with the completed evaluation of an insect-derived food product, without raising any safety concerns for human intake, except regarding allergenicity. Its consumption might induce primary sensitisation and allergic reactions to new IgE-binding proteins and/or cause allergic reactions in subjects with an allergy to crustaceans and/or house dust mites due to the presence of pan-allergens [4].

This presentation will provide an overview of insects as alternative protein sources, the legislative framework currently in place across Europe, the key elements required for allergenicity assessment of novel foods, the available tools for allergenicity prediction and the most advanced technologies for food allergen detection and characterisation.

**Acknowledgments**
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**References**
1. Teixeira, C.S.S. *et al.*, 2023. Food Research International 169: 112847.
2. Pali-Schöll, I. *et al.*, 2019. Trends in Food Science & Technology 84: 45-48.
3. Turck, D. *et al.*, 2016. EFSA Journal 14 : 4594.
4. Turck, D. *et al.*, 2021. EFSA Journal 19: 6343.

## THE ROLE OF SKIN BARRIER IN DEVELOPING IGE-MEDIATED FOOD ALLERGY

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Immune-globulin E (IgE)-mediated food allergy is characterized by a variety of clinical entities within the gastrointestinal tract, skin and lungs, and systemically as anaphylaxis. The default response to food antigens, which is antigen-specific immune tolerance, requires exposure to the antigen and is already initiated during pregnancy. After birth, tolerance is mostly acquired in the gut after oral ingestion of dietary proteins, whilst exposure to these same proteins via the skin, especially when it is inflamed and has a disrupted barrier, can lead to allergic sensitization. This is often the case in children with atopic dermatitis. The crosstalk between the skin and the gut, which is involved in the induction of food allergy, is still incompletely understood. We will focus on mechanisms underlying allergic sensitization (to food antigens) via the skin, leading to gastrointestinal inflammation, and the development of IgE-mediated food allergy. Next to the role of skin to gut axis, also the role of gut to skin axis will be shortly discussed. Better understanding of these processes will eventually help to develop new preventive and therapeutic strategies in children to avoid the development of food allergy.

## ALLERGEN-SPECIFIC B CELL RESPONSES IN FOOD ALLERGY

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A hallmark of allergic sensitization is the induction of allergen-specific IgE responses. While this phenomenon has been the focus of extensive investigation for decades, the exact mechanisms orchestrating IgE production remain only partially understood.

Despite the brief half-life of IgE in circulation, numerous individuals with food allergies, especially those sensitive to peanuts, tree nuts, fish, or shellfish, exhibit lifelong sensitization to these trigger allergens, even as they diligently attempt to avoid consuming such foods. This underscores the existence of an enduring allergen-specific immune memory compartment in these patients, which sustains IgE production over time. B cells and their offspring, as the origin of allergen-specific antibodies, hold a pivotal role in both the development and maintenance of IgE-mediated allergies. The cellular components within the allergen-specific immune memory compartment encompass allergen-specific memory B cells, antibody-secreting cells (including short-lived plasma cells (SLPCs) and long-lived plasma cells (LLPCs)), along with allergen-specific $CD4^+$ memory T cells that act as vital regulators of allergic responses.

During this presentation, I will delve into various modes of class-switch recombination (CSR) that lead to the formation of IgE-switched B cells, alongside highlighting the additional insights gained from characterizing allergen-specific B cells. Furthermore, I will delve into the features of $IgE^+$ plasma cells (PCs), the mechanisms governing the differentiation and survival of $IgE^+$ PCs, and their distribution throughout the body.

## COULD THE REAL TREG PLEASE STAND UP - THE HUNT FOR THE ELUSIVE PEANUT ALLERGY-SUPPRESSING T CELL SUBSET

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The aim of this talk is to review the current knowledge about regulatory T cells (Tregs) that may underlie natural tolerance to foods and could be induced by allergy immunotherapy. In 1986, T cell immunology has been changed forever by the seminal paper by Mosmann *et al.* [1], who described the existence of two different types of T helper cells that could be distinguished by their distinct profiles of secreted cytokines. These types were labelled Th1 and Th2. Subsequently, other Th subsets such as Th17, Th9, Th22 and TFH were discovered.

Classically, allergic diseases have long been attributed to dysregulated, excessive Th2 activity. Nevertheless, the more recent discovery of $CRTH2^{+}$ $CD161^{high}$ $CD49d^{high}$ Th2A by Wambre *et al.* [2] suggested that the Th2 population is not homogeneous. Lastly, the identification of Tfh13 that secrete IL4, IL5 and IL13 and are required for the production of high-affinity IgE and drive anaphylaxis added even more complexity to the picture. Nevertheless, the universe of Th2 cells seems crystal-clear compared to the multitude of Treg subsets that have been identified along the years. In 1971, Gershon and Kondo [3] described infectious immunological tolerance mediated by T cells. Subsequently, oral antigen-induced Th3, IL10-dependent Tr1 and most importantly multiple $CD25^{+}$ Tegs subsets were also discovered.

Nevertheless, the development of single cell whole genome analysis led to the emergence of many more novel Th subsets that emerge or are modulated during immunotherapy of peanut allergy, for example. A mathematical immunology approach, looking at the multiplicity of subsets described in the literature, will be used to present the current knowledge about these emerging T cell subsets and outline the missing gaps that still persist.

**References**

1. Mosmann, T.R. *et al.*, 1986. The Journal of Immunology 136: 2348-2357.
2. Wambre, E. *et al.*, 2017. Science Translational Medicine 9: eaam9171.
3. Gershon, R.K. and Kondo, K., 1971. Immunology 21: 903-914.

## SPEED PRESENTATIONS

*Short presentation by selected poster presenters*

The abstracts can be found in the section ‘Poster abstracts’ (pages 52-86).

P1
Long-term tolerance to cashew nut after low dose oral immunotherapy in preschool-aged children
**Lieke Barten**
Paediatric Allergy Treatment Centre, Deventer Hospital and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, the Netherlands

P5
Neglected wheat species as a source of hypoallergenic lines: Unlocking the potential through genetic exploration
**Lisa Call**
Department of Crop Sciences, University of Natural Resources and Life Sciences, Austria

P17
Molecular dynamics simulation of allergens in food: An in silico approach
**Amin Mousavi Khaneghah**
Department of Fruit and Vegetable Product Technology, Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology, State Research Institute, Poland

P21
Developing an ip animal model to predict sensitising capacity of novel food
**Behnaz Shafie**

## SESSION 2
## FOOD ALLERGEN MANAGEMENT

*How can we assure safe food choices? To answer this question, we need to know where the risks are, what influences food choices, and what we actually eat!*

### EXPECT THE UNEXPECTED – FOOD TRENDS AND CHALLENGES IN ALLERGEN MANAGEMENT

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The past few years we have seen some significant changes in food trends. One of the major trends is of course ‘plant-based everything’ – from plant-based alternatives for dairy products, meat products or any other product of animal origin. Some of these products are based mainly on pulses like soy, or cereals or nuts. ‘Superfoods’ is another trend, e.g., fermented products, green tea, berries, seeds, nuts, ingredients like hibiscus, cannabis, matcha or ancient grains like teff, spelt, sorghum. On top of that ‘sustainability’, ‘free from’ or ‘organic’ continue to be trends. The COVID pandemic has put further emphasis on ‘health’ and ‘immunity’.

So, food consumption patterns are definitely changing. As a result, we also see a change in the exposure to food contaminants like allergens or natural toxins. The use of soy or lupin means that these food allergens need to be labelled. Vegan alternatives for cheese can be based on nuts, which are also food allergens. Of course, a higher consumption of cereal products might lead to a higher exposure of natural toxins. Although oat is considered to be gluten free, contaminations with barley or wheat are possible and therefore a risk with respect to gluten.

Food products based on insects are also a new food trend and might have some risks with respect to food allergies. The EFSA concluded that the consumption of such products may cause allergic reactions to individuals through sensitization to insect proteins, as well as via cross-reactivity of insect protein in individuals allergic to crustaceans, molluscs, and dust mites. Moreover, allergens from the feed (e.g., soy, gluten) can end up in these novel foods, since insects are consumed alongside their intestinal tract.

Therefore, expect the unexpected. Food trends might introduce unexpected allergens and natural toxins from unusual sources as well as change the total contaminant exposure due to the changes in consumption. This of course is a new challenge for allergen management in particular and food safety in general.

## GLOBAL PATTERNS IN FOOD-INDUCED ANAPHYLAXIS

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Anaphylaxis is best described as a serious systemic hypersensitivity reaction that is usually rapid in onset and may cause death. Severe anaphylaxis is characterised by potentially life-threatening compromise in airway, breathing and/or the circulation [1].The incidence of anaphylaxis is probably increasing but fatal anaphylaxis remains rare. The most common cause of anaphylaxis are medications, however, in children food is the most common trigger [2,3].

Food supply increasingly involves supply chains spanning across multiple countries. The Codex Alimentarius (often abbreviated to Codex) is a set of international food standards, guidelines, and codes of practice established by the Food and Agricultural Organization of the United Nations and World Health Organization to facilitate the safety of global trade in food supply. Currently, the Codex requires disclosure for ingredients relating to 8 food groups: cereals containing gluten, crustaceans (such as prawns, crabs and lobsters), egg, fish, peanut and soybean, milk, and tree nuts; sulfites (where present at concentrations of ≥10 mg/kg) must also be declared [4]. Under European law, an additional 5 allergens are also included: celery, lupin, molluscs (such as mussels and oysters), mustard, sesame. The Codex list includes food allergens that are generally considered to cause over 90% of food-induced allergic reactions in most regions. However, anaphylaxis has been reported to almost all foods, and there are significant geographic differences in the prevalence of allergen-specific food allergies worldwide [5], presumably as a result of differences in dietary consumption and/or exposure. Some countries/regions therefore include additional allergens that must be declared on food labels [6].

There are increasing global data relating to prevalence of food allergy and food-induced anaphylaxis; however, this is often based on surrogate measures of sensitization rather than objective symptoms at food challenge. Prevalence data should ideally be derived from unselected populations, but this often results in very small numbers of individuals allergic to a specific food and thus a high level of uncertainty over the resulting estimated prevalence data generated. To address this evidence gap, a systematic review was recently conducted assessing geographic differences in the proportion of anaphylaxis triggered by specific foods, by evaluating the causes of food-induced anaphylaxis presenting to Emergency Departments in different regions [7]. Sixty-five studies (encompassing 41 countries and all 6 CODEX regions) were included. Significant regional variations in the most common triggers of food anaphylaxis were seen; however, in general, there was good agreement between local legislative requirements for allergen disclosure and the most common allergens for each region or nation. Local legislation for allergen disclosure generally reflects those allergens commonly responsible for food anaphylaxis. Cow's milk and crustaceans appear to cause a higher proportion of anaphylaxis compared to peanut in some regions. These data are useful to inform global and regional strategies to reduce the risk of food-induced anaphylaxis.

### References

1. Cardona, V. *et al.*, 2020. World Allergy Organization Journal 13: 100472.
2. Brown, S.G. *et al.*, 2013. Journal of Allergy and Clinical Immunology 132: 1141-1149.e5.
3. Turner, P.J. and Campbell, D.E., 2016. Current Opinion in Allergy and Clinical Immunology 16: 441-50.
4. Food and Agriculture Organization of the United Nations; World Health Organization; Codex Alimentarius Commission General standard for the labelling of prepackaged foods. 1985. http://www.fao.org/ag/humannutrition/32500-0b35b42f09191500b210bf4812e762381.pdf
5. Prescott, S.L. *et al.*, 2013. World Allergy Organization Journal 6 :21.
6. Allen, K.J. *et al.*, 2014. World Allergy Organization Journal 7: 10.
7. Baseggio Conrado, A. *et al.*, 2021. Journal of Allergy and Clinical Immunology 148: 1515-1525.e3.

## ACCIDENTAL FOOD-ALLERGIC REACTIONS: ARE PEOPLE TAKING RISKS OR SIMPLY MISUNDERSTANDING ALLERGEN INFORMATION?

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Food hypersensitivity (FHS) includes food allergy, food intolerance and coeliac disease. Eating a food you are sensitive to, can result in an adverse reaction with unpleasant and sometimes life-threatening symptoms. Management of FHS therefore involves individuals being aware of risks and determining if a food is safe to eat. Children, adolescents, adults, and parents of children with FHS invest a large amount of time and resource in managing the risks associated with an adverse reaction, which includes always checking allergen information before eating. Research over the last twenty years has demonstrated that this burden, along with the unpredictable nature of FHS reactions, has an impact on quality of life [1-4]. A study by Knibb *et al.* [4] found that up to 50% of adults or parents reported they are their children had been served a food containing an allergen in the last 12 months, despite declaring they had a FHS to the allergen. In addition, 40% of adults, 76% of parents of children with FHS, and 79% of children themselves reported at least one reaction in the last 12 months. It is therefore important to understand the nature of these reactions in order to develop strategies to reduce the risk of their occurrence.

This presentation will report on a study carried out in the UK. to investigate reasons for reactions or near misses to food or drink in adults and children with FHS. The study aimed to characterise reactions and near misses, the type of business the food was purchased from and what type of food packaging was involved. It also aimed to understand the journey which resulted in a reaction or near miss and identify the reported cause/s of reactions and near misses. This presentation will provide insights into the most common places of purchase of food causing reactions and near misses and differences in the journey which results in a reaction or near miss to packaged food compared to food sold loose. The presentation will explore whether communication about FHS requirements from the consumer or the food business make a difference and whether causes of reactions are different to causes for near misses. The presentation will conclude with how these results can be used to reduce risk of reactions and near misses in consumers with FHS.

**References**

1. Golding, M.A. *et al.*, 2021. Annals of Allergy, Asthma & Immunology 127: 536-547.e3.
2. Golding, M.A. *et al.*, 2023. Pediatric Allergy and Immunology 33: e13743.
3. Knibb, R. *et al.*, 2021. https://www.food.gov.uk/research/food-hypersensitivity/food-sensitive-study-quality-of-life-wave-1-report
4. Knibb, R. *et al.*, 2022. https://www.food.gov.uk/research/food-sensitive-study-wave-two-survey

## TO EAT OR NOT TO EAT: ADHERENCE TO DIETARY ADVICE AFTER FOOD CHALLENGES

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A food challenge is the gold standard to confirm or rule out food allergy. Furthermore, it gives information about the severity of the food allergy and the threshold. The outcome of a food challenge is used to give patients dietary advice about reintroduction or avoidance of the food.

After a negative food challenge, patients are advised to reintroduce the food in their daily diet. This is important because it helps to reduce unnecessary restrictions in the diet which are shown to be associated with nutritional deficiencies, increased costs, and a negative impact on quality of life. Moreover, the importance of exposure in decreasing the risk of developing food allergy has been demonstrated in children. Remarkably, patients frequently do not succeed in reintroducing the food after negative food challenge. Studies in children and adults show that around 40% fail to reintroduce the food. Patient-reported reasons for reintroduction failure are (atypical) symptoms during reintroduction, ongoing fear for an allergic reaction, being not convinced by the challenge test result, aversion, no need to eat the food, habit of avoiding the food and having family members who also eliminate the food. Several factors are associated with a higher chance of reintroduction failure, for example being a girl, lower age, not receiving advice about food reintroduction, symptoms occurring during food challenge, symptoms during reintroduction, lower quality of life, higher state anxiety and the type of allergen.

After a positive food challenge, dietary avoidance of the culprit food is the key intervention. Adherence to the dietary advice is important to prevent accidental allergic reactions. Studies in food allergic children, adolescents and adults show that patients often fail to adhere to dietary advice to avoid the culprit foods. In adults, only one third of the patients with a positive food challenge adhered to the advised diet. Variables associated with non-adherence were misremembering dietary advice, impaired health-related quality of life on the domain ‘emotional impact’’ and the need for dietary change after the food challenge.

The low frequency of dietary adherence is a major concern because of the risk of unnecessary product avoidance and social impairment in case of a stricter diet than advised and because of the risk of accidental allergic reactions in case of a less strict diet. This stresses the need for more patient-tailored care in which each patient receives counselling and education to manage the reintroduction or elimination of the culprit food(s).

## HOW MUCH WE EAT – FOOD INTAKE DATA IN FOOD ALLERGEN RISK ASSESSMENT

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Food intake data is a crucial input parameter in food allergen risk assessment. Data from general population food consumption surveys are suitable for use in food allergen risk assessment [1], provided an appropriate choice is made regarding the food intake value derived from such surveys. For example, as food allergic reactions generally develop within half an hour, the amount of food consumed during one meal is to be used [2]. Blom *et al.* [3,4] showed that the p50 of the general population single meal intake of a food is most appropriate for deterministic food allergen risk assessment and calculation of action levels for precautionary allergen labelling. Based on these studies, we developed further guidance for the selection of appropriate food intake data for food allergen risk assessment and calculation of action levels.

*Can data from one country be used for another country to bridge potential gaps present in national food consumption survey data?*
National food consumption survey data should only be used for another country when risk assessment outcomes for comparable food product groups between the countries are fairly similar. Therefore, Meima *et al.* [5] conducted a systematic comparison of risk assessment outcomes for a wide variety of food groups, using data from both the United States and the Netherlands population food consumption surveys. Risks were calculated for 14 allergenic foods for 9 allergen concentrations (1-10,000 ppm) to assess comparability. The results showed that depending on the assumed concentration, the risk assessment outcomes for 20% of the food groups (10 out of 49) varied considerably. Due to this relatively high number of potential risk differences, it was concluded that food intake data from the US and the Netherlands cannot be used interchangeably. To enable risk assessments that encompass scenarios for multiple countries, the authors recommended the development and use of a food intake dataset based on the highest intake levels for each food group in the respective countries. This approach would facilitate efforts in risk management and harmonization.

*The TNO Food Intake Guidance tool*
Using data from US, Dutch and combined northwestern Europe food consumption survey data, we developed a user-friendly tool for the selection of appropriate food intake values for deterministic food allergen risk assessment and calculation of action levels for precautionary allergen labelling. A stepwise approach guides the user in identifying the appropriate food group with the corresponding region-specific or generic food intake amount (reference amount, RfA). The presentation will demonstrate how the tool was constructed and the underlying data used to select RfAs.

**References**
1. Blom, W.M. *et al.*, 2020. Food and Chemical Toxicology 146: 111781.
2. Houben, G.F. *et al.*, 2020. Chemical Toxicology 146: 111831.
3. Blom, W.M. *et al.*, 2019. Food and Chemical Toxicology 125: 413-421.
4. Blom, W.M. et al., 2023. Submitted (The 50th percentile of the general population single eating occasion intake of food is optimal for the calculation of action levels for precautionary allergen labelling).
5. Meima, M.Y. *et al.*, 2021. Food and Chemical Toxicology 150: 112006.

## SESSION 3
## FOOD ALLERGY MANAGEMENT

*How can we verify food safety? Once we know what we eat, we need to know what's in it!*

### AOAC FOOD ALLERGENS WORKING GROUP: DEVELOPMENT OF GUIDANCE FOR METHOD DEVELOPERS AND END USERS

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Since 2021, the AOAC Food Allergens Working Group has been in the process of developing guidance on food allergen immunoassay validation. The objective of these efforts was to generate comprehensive, consensus recommendations for developers of both qualitative and quantitative food allergen methods to use in various types of method validations (e.g., single laboratory and collaborative studies). The guidance will also form the basis on which AOAC will evaluate submitted method validation data. The recommendations are built upon prior work on food allergens conducted by members of the AOAC community as well as long standing AOAC practices for collaborative validation studies. Key emerging issues addressed in the guidance include the following: reporting unit requirements, incurred test material preparation guidelines and use requirements, procedures for estimation of limits of detection and quantification, and single laboratory study designs to estimate intermediate precision and repeatability. The working group believes the guidance will be an invaluable resource for the assessment of food allergen method performance both within the AOAC approvals process and more broadly. As the current document only addresses recommendations for method developers, subsequent work will focus on guidance for end users of food allergen methods.

## STANDARDIZATION OF A REFERENCE PROTOTYPE-BASED METHOD TO QUANTIFY FOOD ALLERGENS IN COMPLEX FOODS AND COMPLIANCE WITH REFERENCE DOSES: AN OUTCOME OF THE ThRALL PROJECT

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Food allergy is considered a major safety issue for the life-threatening reactions that the ingestion of tiny amounts of allergens can trigger in sensitive consumers. On this regard, efforts to protect allergic population have been put in place at both levels: (i) establishing reactivity thresholds for each individual allergen; and (ii) developing sensitive and reproducible analytical methodologies to detect and quantify even minute amounts of allergenic ingredients in foods, to verify compliance with the reference doses recommended. Although European legislation with regulation 1169/2011 mandated the labelling of 14 allergenic ingredients whenever intentionally introduced into a food, a risk of detecting allergens in foods expected to be allergen free is likely to exist due to cross-contamination occurring along the same food supply chain. The need to guarantee a higher level of protection of allergic consumers from an accidental cross-contamination, has prompted food industries to make excessive use of precautionary allergen labelling (PAL), Also, risk assessment workflows have been adopted by the food industries to limit such risk. Despite the progress done in the specific field, food allergy research still faces drawbacks and unmet challenges due to the extreme variability of individual sensitivity to the different allergens, the establishment of threshold levels, the absence of validated and standardised analytical methods for allergen quantification in foods. These represent the main causes for the absence of a regulatory framework for the management of hidden allergens. In order to overcome these drawbacks, analytical methods with challenging sensitivities, basing of trustful and reproducible results, have been developed over the last two decades using antibody recognition, DNA or mass spectrometry detection for allergens determination, although only few of them have undertaken a validation study. The recent ThRAll project funded by EFSA [1] was aimed at achieving two main objectives: (i) detection and quantification of allergens in foods; and (ii) minimum eliciting doses in food-allergic individuals. The development of a prototype method based on mass spectrometry for the quantitative determination of a total of six allergens in a complex food matrix was in this project complemented by the second objective to support the development and curation of data on oral food challenges, to be further used to define thresholds and minimum eliciting doses. All the key steps in method development and validation of the multi-target prototype LC-MS/MS method for the quantification of six allergens in chocolate, chosen as complex food model, will be illustrated in this note [2,3]. Final results and performance of the method will be presented and compliance with the reference doses recommended by the FAO/WHO expert consultation working group for the different allergens, will be discussed.

**Acknowledgments**
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**References**
1. Mills, E.N.C. *et al.*, 2019. Journal of AOAC International 102: 1346.
2. Henrottin, J. *et al.*, 2023. Food Control 143: 109256.
3. Pilolli, R. *et al.*, 2023. Analytical and Bioanalytical Chemistry, in press.

## RELIABLE IMMUNOSENSING PLATFORMS FOR THE MULTIPLEXED DETERMINATION OF MAJOR ALLERGENS

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The increasing prevalence of allergic diseases has necessitated the development of sensitive and reliable techniques for the detection and quantification of major allergens in food samples. This communication presents a comprehensive view of immunosensing platforms tailored to address the urgent need for multiplexed determination of major allergens, aiming to enhance safety measures and improve the quality of life for individuals with allergies. Our work focuses on advancements in multiplexed immunoassay-based methodologies, including lateral flow assays (LFAs) and micro enzyme-linked immunosorbent assays. Notably, the emergence of nanotechnology and microfluidics has enabled the development of innovative allergen detection platforms with enhanced sensitivity, selectivity, and reduced analysis time. Point-of-care applications and smartphone-based readout systems are also discussed, offering rapid on-site allergen testing potential.

Furthermore, a critical assessment of the challenges and limitations faced by current immunosensing platforms is provided, paving the way for future research and technological improvements. Key considerations include matrix interference, cross-reactivity, and storage stability, which must be addressed to establish more reliable detection systems. The comprehensive analysis of immunosensing platforms yields valuable insights for researchers and industry professionals seeking to implement robust and multiplexed allergen detection strategies, ultimately contributing to advancing public health and safety globally.

## FOOD ALLERGEN DETECTION, A COMPLETE AND VERSATILE SOLUTION COVERING ALL RAPID TESTING METHODS

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Food allergens are becoming an increasing concern in modern population, considering the high percentage of affected patients, as well as the new emerging allergens related with the use of innovative ingredients in food processing. Consequently, the possibility of providing a comprehensive solution for allergen detection is a must for food allergen management in the food industry and in agro-food laboratories. The key topics are: (i) new and emerging allergens in the food industry; (ii) allergens legislation – new challenges; (iii) different techniques for food allergen detection; and (iv) the Gold Standard Diagnostics solution – covering the entire allergen management process from an analytical point of view.

## EXPANDING THE SCOPE OF A ROUTINE LC-MS/MS APPROACH TO ALLERGENS TESTING

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Food allergies are the leading cause of anaphylaxis, an acute, potentially deadly allergic reaction. The prevalence and severity of food allergies are rising, with approximately 150 million people suffering from food allergies worldwide. Presently, there is no cure for food allergies, and sufferers must rely on the correct labelling of foods to avoid consuming allergens. Hence, developing sensitive and accurate analytical methods to screen for the presence of allergens in food products is necessary to prevent potentially life-threatening health problems for allergy sufferers. SCIEX vMethod application for food allergen testing previously provided a workflow for sample preparation and LC-MS/MS detection of 13 distinct allergens, including egg, milk, almond, Brazil nut, cashew, hazelnut, pine nut, pistachio, pecan, walnut, peanut, soy and sesame. In addition, a highly selective and sensitive LC-MS/MS method was developed to quantitate wheat gluten proteins and to identify what are the major grains in the food matrix at the same time by monitoring ray, barley, and oats unique peptides in the bakery, fermented beverage and baby formula products.

**PEPTIDES, PROTEINS, AND CONVERSION FACTORS: HOW DO THEY AFFECT MY MEASUREMENT UNCERTAINTY?**

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The Joint Research Centre of the European Commission developed a reference method based on liquid chromatography mass spectrometry for the determination of total cow's milk protein content in food. The sample treatment to be applied consists of a protein extraction from baked cookie samples; spiking with several target labelled peptides; and a digestion and clean-up before analysis. A total of 11 peptides are quantified to characterise the 5 constituent proteins. Applying a set of well-defined conversion factors, the total cow's milk protein value can be derived from the results obtained for (i) each individual peptide, (ii) each constituent proteins, (iii) the total casein content, or (iv) the sum of the 5 proteins investigated. This presentation will discuss the various measurement uncertainties calculated.

## MULTI-ALLERGEN QUANTIFICATION IN FOOD USING CONCATEMER-BASED ISOTOPE DILUTION MASS SPECTROMETRY: A COLLABORATIVE STUDY

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Food allergen analysis is an essential tool for control and the development of a risk-based approach to allergen management. Robust, specific, and sensitive detection methods are needed to protect consumers with allergies and to guarantee correct food labelling. During the last decade, mass spectrometry has become a method of choice for allergen analysis. Mass spectrometry relies primarily on the analysis of specific peptides obtained by enzymatic digestion of proteins extracted from a sample. A series of mass spectrometry-based methods targeting single or multiple food allergen(s) have recently been developed and described in the scientific literature. In some cases, the performance of these methods, including their sensitivity and accuracy, has been evaluated and validated in single laboratories.

Inter-laboratory validation is a necessary step towards harmonization in food allergen analysis. However, efforts aimed at harmonization among laboratories, both in terms of the analytical pipelines and the obtained results, are lacking. Although several inter-laboratory studies on methods using techniques based on immunoassays or DNA detection have been published, to the best of our knowledge, no inter-laboratory study using a mass spectrometry-based method has been reported. The ‘Allersens’ project was a 4-year research programme funded by the Belgian Federal Public Service Health, Food Chain Safety and Environment in 2016. During the course of this programme, we have developed and validated a mass spectrometry-based method for the quantification of four major food allergens (egg, milk, peanuts, and hazelnuts) in processed food products. The quantification strategy was based on standard addition method, and stable isotope-labelled concatemer was used as the internal standard.

To demonstrate the feasibility of harmonization among analytical laboratories for the analysis of food allergens by mass spectrometry, we invited laboratories across Europe to apply our method. In total, nine laboratories participated in the collaborative study. The analytical procedure, blank and incurred cookie matrices, standards and stable isotope-labelled concatemer internal standard were provided to the participants. The collected quantification results allowed to evaluate the selectivity, sensitivity, trueness, and precision of the method. The results of this inter-laboratory collaboration demonstrated the potential of our mass spectrometry method and quantification strategy for food allergen analysis. Harmonization and effective methods are essential for allergen management and to guarantee safe food for consumers with allergies.

## COMPARATIVE STUDY OF EGG AND CELERY ALLERGEN ELISA AND DNA KITS IN DIFFERENT FOOD MATRICES

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Allergen management differs considerably from other food safety issues. What makes allergen risk assessment uniquely different and complex, is that a very low level unintended presence in food products, can already evoke a harmful physiological response in allergic consumers. Using test methods for self-control by producers in the food production chain has become common practice. Based on the results of these tests, producers can, when necessary, respond quickly to avoid potential safety or quality risks. However, there are issues in the quality performance of detection methods, especially when used with complex matrices or processed foods. Proper allergen detection is very important when precautionary allergen labelling will be implemented based on references doses and allergen detection methods need to be sensitive and reliable enough to reveal allergens just above the action limit.

Therefore, Wageningen Food Safety Research together with food industry, commercial labs, and kit developers, will evaluate if commercially available ELISA and/or DNA test kits for egg, soya, milk, and celery are fit for purpose. Furthermore, the results will be compared to mass spectrometry analysis. In this presentation, results of the comparative studies for the target allergen egg and celery are reported. For egg seven ELISA test kits, and for celery four DNA kits were compared. Relevant food matrices with cognate blanks, spiked and incurred samples were measured for both allergenic food ingredients. The results of this comparative study on egg ELISA and celery DNA test kits and the comparison with LC-MS analysis will be presented. These results are of interest for people involved in allergen management, test development and allergen analysis.

## SESSION 4
## FOOD ALLERGY MANAGEMENT AND THERAPY

*Characterising and curing the patient, where are we with that? And in the end… what is the food allergic patient's perspective on food allergen and allergy management?*

### CORE OUTCOME SET FOR IGE MEDIATED FOOD ALLERGY: WHAT TO MEASURE?

A. Demidova, K.P. Drewitz, P. Kimkool, J. Genuneit, R.J. Boyle, C. Apfelbacher and **Daniel Munblit** on behalf of COMFA Consortium

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The Core Outcome Measures for Food Allergy (COMFA) initiative is a comprehensive effort targeted at homogenising outcomes for clinical trials and observational studies of interventions for IgE-mediated food allergy (FA). Food allergy presents a significant global health concern with prevalence and severity have substantial personal and societal implications, including diminished quality of life, increased healthcare costs, and heightened anxiety about food-related activities. Given the diversity of intervention strategies that have been explored for managing FA, inconsistencies in reported outcomes have often complicated comparisons across different studies and meta-analyses. Therefore, the development of a Core Outcome Set (COS) aims to address these challenges, ensuring more reliable comparisons between different intervention strategies.

The project implemented a comprehensive and systematic approach to formulate the COS. It began with a thorough review of existing literature and a subsequent classification of FA outcomes, covering a broad range of studies and interventions. The data gathered from this review served as the foundation for a two-round online modified Delphi process which involved people with lived experience, healthcare professionals and researchers. Following the Delphi process, a hybrid consensus meeting was held with all stakeholders to finalise the COS, ensuring its wide acceptance and applicability.

The review and classification of outcomes produced an initial list of 39 potential outcomes. This list was further refined after iterative discussions with various stakeholders, including patients, caregivers, clinicians, and researchers, which led to a more focused selection of 13 outcomes. A total of 778 participants from 52 different countries were involved in the Delphi process with 442 involved in both Delphi rounds with results informing consensus meeting. From this process, two outcomes – 'allergic symptoms' and 'quality of life' – reached consensus for inclusion in the final COS.

In conclusion, the COMFA initiative has provided a standardised COS for IgE-mediated FA clinical trials and observational studies of interventions. By prioritising outcomes that are meaningful to people with lived experience, healthcare professionals and researchers, this initiative effectively aligns future research with the actual needs of those directly impacted by FA.

## IMMUNE SIGNATURES DURING ORAL FOOD CHALLENGES

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Diagnostic food challenges are the gold standard to diagnose food allergy. They are also important to monitor the patients´ progression, both the natural progression and the impact of therapeutic intervention, such as oral immunotherapy, on threshold dose reactivity. Food challenges are an important burden for the patient as well as the clinical centers, where capacities are limited due to rising demands. So far, ex-vivo biomarkers that would allow to predict the outcome of food challenges are evasive.

Detailed knowledge of the complex Th2 immune pathways underlying clinical reactivity is key for the development of novel biomarkers and precision diagnosis in PA. Recent advances in immunological endotyping using cutting-edge technologies in flow and mass cytometry have opened new routes for dissecting immune responses in food allergy. Studies based on ex-vivo immune cell stimulations with food allergens demonstrated the engagement of particular T cell subtypes as well as others, such as cells of the innate immune system, into the allergic response. However, immune insights derived from ex-vivo stimulations cannot be directly compared to the dynamic situation of evolving clinical symptoms during food allergen exposure. Recent studies focused on investigating acute food allergy events, using blood samples taken during food changes with food-allergic patients. Those studies pointed to relevant immune cell changes in the peripheral blood over food allergen exposure. As a first study of its kind, we directly analyzed longitudinal cellular immune profiles in-vivo before and after food allergen challenge, providing a comprehensive overview on temporal changes in deep immune signatures. Those findings will be discussed in the light of the current literature evidence for bridging to their potential basis in predictive biomarker discovery and monitoring food allergy outcome.

## HMOS AND IMMUNOMODULATION: POTENTIAL FOR THE MANAGEMENT OF ALLERGIC DISORDERS?

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Our body is attacked continuously by many different danger signals. A resilient and effective immune system is essential in order to protect and repair. Unbalanced immune responsiveness seems to play a significant role in the enormous increase in non-communicable diseases (NCDs) including allergies. It is essential to understand and develop new avenues to manage allergic disorders. Early life programming including proper education of our immune system has been recognized as a promising approach and might lead to unique opportunities for both prevention as well as novel therapies of immune related disorders such as allergies at all age categories. One of the lessons we learned from human milk is the unique immunomodulating properties of ingredients present in this milk. Some of these might play a significant role in educating and training our immune system which might help to prevent and/or protect against allergies including food allergy. Unique combinations of free amino acids, lipids, micro-vesicles, specific epitopes, human milk oligosaccharides (HMOS), microbes,… are fascinating examples of human milk ingredients influencing our immune system relevant for both prevention but also management of allergic disorders.

As an example, the awareness of the importance of a diverse microbiome in immune-regulation and as a consequence impact on immune related disorders such as allergies is growing exponentially. After birth, the development of a ‘healthy’ gut microbiome is considered to play an important role in immune development. HMOS and even some unique microbes are transferred by the mother through the breastmilk to the child. It has been shown that the HMOS can induce a gut microbiome that is at least in part associated with a healthy and balanced immune system (the prebiotic function). In addition to indirect effects of the HMOS on the immune system via microbiome changes HMOS can affect immune cells in a direct fashion as well. Many research groups are currently in search for the unique mechanism involved and show for example the involvement of unique receptors and lectins/galectins that are at least in part responsible for these direct immune effects.

Both pharma as well as specialized-nutrition companies do see the highly relevance of HMOS to manage some immune related disorders such as allergies. However more research is needed in order to validate and understand the uniqueness of HMOS in both classical pharma approaches as well as specialised nutrition aimed at allergy management such as immunotherapies.

## GOOD FOOD DOES EXIST: IMPACT OF SPECIFIC FOOD COMPONENTS (DIETARY FIBRE AND OMEGA-3 PUFAs) ON ALLERGY

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The prevalence of adverse health conditions that can be associated to allergic mechanisms will reach near-pandemic proportions, if the EAACI-prognosis, that by 2025 more than 50% of all Europeans will suffer from at least one type of allergy, becomes reality [1]. Food allergies will form a significant fraction of this, not only because of absolute numbers of sufferers, but also because of the potential severity of reactions.

For decades, strict avoidance of offending allergens has been the only remedy for those affected. However, with such a magnitude of prevalence, (research) efforts can no longer be mainly focused on attempts to minimize the chance of accidental contact between allergens and allergy sufferers but should also develop attempts to prevent the adverse health condition to develop at all. Is it realistic to expect that the development of (food) allergies can be prevented? Yes it is, as e.g., the LEAP [2], LEAP-ON [3], EAT [4] and PETIT [5] studies have demonstrated, and have resulted in paradigm shifts at an unprecedented pace.

Relationships between (quality of) diet and allergies have been established, and better understanding of these may offer additional perspectives to reduce the symptomatic burden and to support strategies as described in the above-mentioned studies. On the one hand, tools to predict the immunological impact of (novel) dietary proteins are being developed. On the other hand, the importance of, for instance, sufficient intake of various types of dietary fibres and of ω-3 poly unsaturated fatty acids is becoming increasingly apparent. Beneficial effects of these compound groups on allergic reactions, and how such possibly works mechanistically, will be discussed.

**References**

1. https://www.veroval.info/-/media/diagnostics/files/knowledge/eaaci_advocacy_manifesto.pdf
2. https://www.nejm.org/doi/full/10.1056/nejmoa1414850
3. https://www.nejm.org/doi/full/10.1056/nejmoa1514209
4. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4852987/
5. https://www.sciencedirect.com/science/article/pii/S0140673616314180?via%3Dihub

## THE POTENTIAL ROLE FOR EPIGENETICS IN THE MANAGEMENT AND TREATMENT OF FOOD ALLERGY

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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. 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 personalized treatment options being rolled out epigenetic modifications might well play a role in monitoring or even predicting the response to tailored therapy. Furthermore, analysis of the epigenome might provide novel targets for therapeutic intervention. miRNA mimics, inhibitors and antisense oligonucleotides are currently evaluated in clinical trials in other diseases, while extracellular vesicles represent future tools for the modulation of the cellular phenotype and responses. Moreover, interactions between the host epigenome and the microbiome are increasingly recognized 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.

## A NEW PERSPECTIVE ON ORAL IMMUNOTHERAPY FOR FOOD ALLERGY, AGE MATTERS?

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The worldwide rising prevalence of food allergy is a major public health concern. Standard care consists of strict allergen avoidance and rescue medication upon accidental exposure. Oral immunotherapy (OIT) is increasingly being studied as a treatment option. While desensitization (an increased reaction threshold) is often reached during OIT, sustained unresponsiveness (SU; clinical non-reactivity after finishing OIT) is not achieved in most patients A limited number of studies have investigated the effectiveness of OIT in preschool-age children (early=e-OIT), showing a much favourable outcome regarding long-term tolerance development. Together with the food allergy prevention studies, which have demonstrated high effectiveness of early oral allergen exposure, the outcomes indicate an early life window of opportunity to achieve SU allowing for unrestricted dietary intake. However, the underlying mechanism of the high effectiveness of e-OIT is not understood. Both cohort and OIT studies indicate early-life immune plasticity. An immature allergic response in the first years of life seems to be a main driver of the immune plasticity, together with a higher tolerogenic immunological state in early years of life. Allergy maturation can probably be effectively disrupted by early intervention, preventing the development of persistent food allergy. Upcoming studies will provide important additional data on the safety, feasibility, and effectiveness of e-OIT.

## UPDATE ON SUBLINGUAL IMMUNOTHERAPY FOR PEANUT ALLERGY

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Oral immunotherapy (OIT) has been shown to successfully desensitize food-allergic patients resulting in the EMA and FDA approval of Palforzia as the first OIT formulation for peanut allergy. However, OIT may not be feasible for all patients due to its difficult administration protocol and/or concerns about the risks of therapy. Sublingual immunotherapy (SLIT) may be a promising alternative to OIT due to its simple administration combined with good efficacy and safety.

Early reports provided proof of concept with SLIT for kiwi, hazelnut, and peach allergies. Blinded studies at the University of North Carolina (UNC), Johns Hopkins University, and through the Consortium for Food Allergy Research (CoFAR) have subsequently demonstrated efficacy and safety with SLIT for peanut allergy. In the most recent study of peanut SLIT at UNC, peanut-allergic children ages 1-11 years were desensitized to a mean cumulative tolerated dose of 2,723 mg of peanut. 70% of children achieved clinically meaningful protection by tolerating over 800 mg of peanut. After discontinuation of peanut SLIT, children were estimated to remain protected above 800 mg for a median of 22 weeks suggesting a need for continued SLIT therapy but also immune modulation that could withstand extended gaps in therapy. Almost 98% of doses were successfully taken with symptoms reported with 4% of doses. The majority of these side effects were transient oral itching and epinephrine-treated side effects were not reported.

Daily treatment with peanut SLIT appears feasible and safe and in peanut-allergic children and provides clinically meaningful desensitization to the majority of children. The peanut SLIT desensitization effect likely requires continued therapy, however clinical protection appears to be maintained through gaps in treatment.

## SESSION 5
## ILSI EUROPE – FOOD ALLERGY TASK FORCE: 25 YEARS DEVELOPING ALLERGEN RISK ASSESSMENT AND MANAGEMENT

*For more than two decades, the Food Allergy Task Force has followed an ambitious aim: to establish consensus among multiple stakeholders on science-based approaches for food allergen risk assessment and management.In this session, an overview of our most recent major projects as well as a roadmap of our new and future activities will be presented.*

### ILSI EUROPE FOOD ALLERGY TASK FORCE: A MULTISTAKEHOLDER APPROACH TO ADDRESS THE RISKS IN FOOD ALLERGEN MANAGEMENT TO PROTECT ALL CONSUMERS

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There is increasing evidence of the rising incidence and prevalence of food allergies worldwide. The Food Allergy Task Force of ILSI Europe has worked on different components of risk assessment ranging from prioritisation and identification of the hazard and its characterisation to consideration of prevalence and exposure and risk characterization. Moving beyond the usual tripartite model (academia, government, and industry), the Task Force has integrated patient organisations and other relevant stakeholders into many of its activities for fostering an international evidence-based consensus on how to assess the risk from allergenic foods.

## ALLERGENICITY ASSESSMENT OF NEW PROTEIN-CONTAINING SOURCES AND INGREDIENTS

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The growing world population, changing dietary habits and increasing pressure on agricultural resources are driving the development of novel foods. These encompass new protein sources (e.g. insects, other protein sources not commonly consumed in the population to which they are marketed) as well as existing protein sources that are produced or used in a form or way different to their familiar presentation. Changes in available foods and a concomitant consumer desire to follow these changes may lead to significant increases in the intake of specific food proteins and may consequently have the potential to increase the prevalence of existing and previously undocumented food allergies, resulting in potential impact on public health. Assessing the allergenicity of new or modified protein-containing food sources or ingredients gives rise to a number of challenges. For example, there is uncertainty about which risk(s) is (are) acceptable, as well as about the assessment criteria for deciding what is considered acceptable or ‘safe’. Furthermore, the methodological tools available to generate the data needed to support any assessment criteria have several gaps.

This presentation will highlight the key findings and conclusions reached by an ILSI Expert Group that reviewed the problem. It summarises the main issues and discusses the interpretation of ‘safe’ in different jurisdictions, describes the societal acceptability of risk in the context of allergenicity and technological feasibility of the parameters and criteria used for risk management decision-making. It proposes a two-part framework for allergenicity assessment. The first part includes sytematic consideration of knowledge and data requirements for comprehensive allergenicity assessment, including societal as well as technological prerequisites. The second part describes application of a generic assessment approach, including a key role for a Threshold of Allergological Concern (TAC), by analogy with the now well-established Threhsold of Toxicological Concern (TTC). Together the two parts serve to highlight the areas that need to be addressed to close the knowledge and data gaps, as well as research priorities for the development and implementation of this framework.

## FOOD ALLERGEN QUANTITATIVE RISK ASSESSMENT (QRA): A MULTISTAKEHOLDER APPROACH TO IMPROVE CONSISENCY IN THE APPLICATION OF QRA BY FOOD BUSINESS OPERATORS

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The landscape of food allergen management is at an inflection point. Globally there is an increased recognition by government agencies, industry and academic experts that quantitative risk assessment (QRA) has utility as a part of allergen management, including as input into decisions on precautionary allergen labelling (PAL). The established practice is that allergen cross-contact is managed in a binary fashion, i.e., either an allergen is potentially present or not. This binary approach, which lacked industry alignment on how it was implemented, has led to a disconnect between the reality of the risks presented by a product that may contain a cross-contact allergen and the management actions taken. Fortunately, the current advent of allergen reference doses and their application via QRA can provide an opportunity to enhance how allergen risks are assessed and therefore managed, but only if these new tools are implemented consistently across and between food supply chains.

Despite the increased maturity of QRA, there is a lack of consensus between stakeholders on practical deployment. This is a significant gap, as the value to allergic consumers of precautionary allergen labelling can only improve if there is consistency between food operators in terms of more harmonized allergen risk assessment approaches. A recent ILSI Europe Expert group gathered a wide range of stakeholders to generate a consensus on the methodologies needed for QRA. This practical guidance enables both proactive and reactive risk assessments in the upstream supply chain, as a part of operational management of cross-contact scenarios, and for assessment of potential production errors/incidents. This consensus and harmonization are needed in order to protect public health and increase the trust of consumers with food allergies in packaged foods, including their labelling, both now and in the future.

## HOW DO WE EFFECTIVELY MANAGE ALLERGENS AND COMMUNICATE RISK OF PRODUCTS WITH PAL TO THE CONSUMER WITH FOOD ALLERGIES?

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The topic of quantitative allergen risk assessment (QRA) is gaining wider acceptance as evidenced by the recently published three report series from the ad hoc Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens. In this presentation, it will be discussed how QRA can be practically applied in the management of operations by food business operators and how this results in better information for food allergic consumers. Next steps and future trends.will also be highlighted.

**FINAL PLENARY SESSION**
**TOWARDS A FOOD ALLERGY-FREE WORLD: LOOKING BACK AND FORWARD**

*What does the (near) future hold?*

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A list of major foods and ingredients known to cause hypersensitivity was included into the Codex General Standard for the Labelling of Packaged Foods (GSLPF) in 1999. There have been many scientific developments in the understanding of food allergens and their management since the original drafting of the GSLPF. Thus, in response to the request from Codex for scientific advice, including current evidence of consumer understanding of allergens, FAO and WHO convened a series of expert meetings to provide scientific advice on this subject.

The first meeting was held in December 2020. Based on the latest scientific evidence, the expert committee identified and used three criteria – prevalence, potency, and severity – for assessing proteins for their potential inclusion or exclusion on a priority food allergen list for Codex.

In March 2021, the expert consultation convened for a second time to establish threshold levels for priority allergenic foods. Knowledge of thresholds constitutes a critical requirement to assessing the risk from allergens, as they are a characteristic of the hazard that allergens present to the food-allergic population. The expert committee concurred that the benchmark dose/probabilistic hazard assessment approach aligned most closely with the requests of the Codex Committees. After extensive discussion, the expert committee reached a consensus on reference doses (RfD) for priority allergenic foods, meeting the criterion for HBGV that they should reflect a range of exposure without appreciable health risk. Through risk assessment, reference doses, based on health-based guidance values for each of the priority allergens were recommended.

In October 2021, FAO and WHO reconvened a third meeting to review and evaluate the evidence in support of precautionary allergen labelling to address unintended allergen presence in foods. The expert committee at the third meeting reviewed the data on the current status and uses of PAL and unanimously agreed that current PAL systems used in many countries needed to be improved as they were neither uniform nor informative and were not consistently risk based on amount and frequency of UAP found in food products. The expert committee also found that current PAL approaches led to widespread PAL that diminished information and value for consumers. The expert committee reviewed again the principles and basis of RfD from the second meeting and reached a consensus that the RfD for each priority allergen, as described by the HBGV and safety objectives, was a valid risk assessment endpoint for determining when sporadic or unexpected UAP posed more than appreciable risk to consumers and needed to be communicated to consumers by PAL.

In November 2022, the fourth meeting addressed whether it was scientifically justifiable that containing certain ingredients derived from priority allergenic foods, such as highly refined oils, could be exempted from mandatory declaration on packaged foods.

In March 2023, the expert committee discussed the thresholds of some food allergens which are not in the priority list.

## PAL-ING AROUND WITH ALLERGENS: ANALYTICAL CONUNDRUMS AND LEGAL PREDICAMENTS – A DIALOGUE

A frank discussion about recommended food allergen threshold levels and implementation.

The ad hoc Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens established threshold levels in its second round, and it recommended ways forward for meaningful precautionary food allergen labelling in its third round.

- How does the food industry perceive this?
- Are there any concerns?
- What are the regulatory implications?
- And can these thresholds be analytically enforced?

Three experts from the food industry, food law and risk assessment/food analysis will discuss the topic and invite questions from the audience.

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## ALLERGENICITY ASSESSMENT OF NOVEL FOODS, THE WAY FORWARD

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The growing world population and increased pressure on agricultural resources are driving a shortage of dietary protein sources. As a result, industry is developing more sustainable novel food protein sources such as insects, algae and duckweed and using new processing techniques. Consumer exposure to these novel or processed proteins, could cause new food allergies, exacerbating a public health issue which is already directly affecting an estimated 20 million Europeans. Introduction of novel foods should not add to the burden of food allergy and this calls for a reliable, harmonised, evidence-based and validated allergenicity risk assessment strategy. The COST (Cooperation in Science and Technology) Action ImpARAS (Improved Allergenicity Risk Assessment Strategy), a four-year networking project, identified gaps in current allergy risk assessment, and proposed new ideas and plans for improving it. The safe introduction of novel and more sustainable food protein sources, while protecting humans from food allergy, calls for a multidisciplinary approach based on an improved understanding of what determines the relative allergenic potency of proteins, novel testing and assessment methodologies, harmonized decision-making criteria, and a clear ranking approach to express the allergenicity of novel product relative to that of existing known allergenic proteins: (from ‘non’/to weakly and to strongly allergenic proteins). Lessons learned from the ImpARAS network and suggestions for future research are taken up by a new European initiative: Marie Curie Doctoral Network ‘ALLPreT’ (Allergenicity Prediction Toolbox).

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**Marit Zuurveld[1],** M.A.P Diks[1], P.C.J. Kiliaan[1], J. Garssen[1,2], G. Folkerts[1], B. van't Land[2,3] and L.E.M. Willemsen[1]

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P1

## Long-term tolerance to cashew nut after low dose oral immunotherapy in preschool-aged children

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Most food allergies develop in early childhood and often persist for life. One of the most potent food allergens is cashew nut (CN), which is known to cause severe allergic reactions. The prevalence of CN allergy is increasing significantly with raising consumption. Current food allergy management consists of dietary avoidance and treatment of allergic symptoms following accidental ingestion. Emerging evidence suggests that Oral Immunotherapy started early in life (e-OIT) is highly effective in inducing long-term tolerance. Since studies on e-OIT for CN are lacking, we are investigating the effectiveness of e-OIT in inducing long-term tolerance in children diagnosed with CN allergy. We performed a sub-group analysis on the preliminary results of an ongoing prospective intervention study on e-OIT in children aged 9-24 months with a wide range of food allergies. Children with proven CN allergy based on sensitization and a positive oral food challenge (OFC) were included in this analysis. Participants received a daily maintenance dose of 300 mg CN protein during 1 year after a build-up phase. Four weeks after stopping e-OIT, tolerance was assessed by an exit OFC. Follow-up phone surveys were performed to assess long-term tolerance. Forty-nine children (median age 17 months, range 9-24 months) with CN allergy are included. To date, 25 children have finished the therapy, 21 are receiving maintenance therapy and 3 children (5.7%) discontinued participation because of aversion and/or mild allergic side effects. The median baseline threshold level of reactivity was 300 mg CN protein (IQR 100-1000 mg). The median Sampson score, indicating severity of allergic symptoms at the baseline OFC, was 2 (range 2-4) on a scale of 1-5. All 25 children who have finished e-OIT became tolerant: they consumed 4.4 grams of CN protein without allergic symptoms during the exit OFC. Follow-up has already been performed in 14 children at an average of 18 months post-treatment. Twelve children (86%) continue weekly/monthly CN consumption. Nine of them (75%) reported no allergic symptoms following ingestion, 3 (25%) reported transient mild symptoms. One child stopped CN consumption 1-year post-treatment due to aversion. Parents of 1 child chose not to introduce CN because of severe eczema. In conclusion, this study is the first to investigate the effectiveness of e-OIT in pre-school aged children with CN allergy. The preliminary results suggest that one year of low-dose e-OIT is highly successful in inducing long-term tolerance. Further follow-up of these children will follow in the coming years.

P2

## Simultaneous quantification of major food allergens using a multiplex immunoassay

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The measurement of food allergens is crucial in food industry safety monitoring. Current food testing methods (e.g., ELISAs) can only test for one allergen at a time and may lack clarity in the specific analyte being measured. Our aim was to develop a high-throughput multiplex immunoassay capable of simultaneously measuring multiple specific allergen proteins (e.g., Gal d 1, Ara h 3) from egg, cow's milk, peanut, tree nuts (hazelnut, walnut, almond and cashew), fish, shellfish, mustard, sesame, soy, and celery. The multiplex array was developed on the Luminex xMAP system. Microspheres coupled to specific monoclonal antibodies were used for allergen capture. Biotinylated specific monoclonal or polyclonal antibodies were used for detection. Allergen reference standards were formulated from highly purified allergen proteins. Validations were performed to determine parameters of linearity, range, limits of quantification and detection, accuracy, and precision. Allergen incurred chocolate bar samples were analysed to confirm detection of allergen from a challenging matrix. Allergen extraction optimisations and recovery assessments were performed using chocolate dessert reference materials incurred with egg, milk, walnut, almond, hazelnut, and peanut. The reference materials were extracted using five different extraction buffers/methods. Intra- and inter-assay variation was established to be <15% and recovery to within 70-130% using purified allergens. The array was successful in detecting egg, milk, peanut, hazelnut, and walnut allergen incurred into chocolate bars at 3, 10, 30, and 100 ppm. Allergen extraction was optimised, and two extraction buffers yielded strong recoveries of 76-147% from the incurred matrix. Interestingly, milk allergens required a different extraction buffer to egg, peanut, and tree nut allergens for optimal extraction. A placebo chocolate dessert matrix verified the array did not produce false-positive results. In conclusion, a quantitative, accurate and precise multiplex immunoassay was validated for the simultaneous detection of 17-major food allergen proteins. The array is an efficient tool for measuring panels of food allergens, with applications for risk assessment and safety monitoring. Additional work is ongoing to optimize and establish recovery data for all other allergens in the array.

P3

**A fast solution for the detection of allergen traces in food samples by LC-HRMS including standardized and automated sample preparation**

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With increasing prevalence of food allergies, even leading to severe symptoms such as anaphylaxis, allergen labelling on food products is regulated by the European Union law. Allergens must be included in the list of ingredients of any product, whereas unintentional presence of allergens is regulated on a voluntary basis by precautionary allergen labelling (PAL), indicated by the statement 'may contain'. Allergic individuals have no choice but to avoid the specific food product. In order to counteract PAL and to be able to meet upcoming thresholds of allergenic ingredients in a reproducible way, an efficient and fast method for allergen detection in food products is required. The given approach is providing a total solution for the screening of different allergens in food samples. It combines sample preparation, analysis by liquid chromatography coupled to high resolution tandem mass spectrometry (LC-HRMS) and software for automated data evaluation. The established sample preparation approach is including protein extraction, enzymatic digestion, and cleanup of peptides. Protein digestion is performed within three hours, requiring less time compared to standard proteomic procedures. The given protocol is suitable for a variety of food products and allergens reducing the complexity of lab work. Mass spectrometric analysis is performed on the Bruker impact II qTOF system, requiring simply one generic method for detection of multiple allergens. The presence of each allergenic component is confirmed by detecting the precursor masses of three specific peptides, resulting from allergenic proteins of the given ingredient. The main advantage of this approach is the multiplexing capability, which enables detection of multiple allergens within one measurement, significantly reducing measurement time per sample compared to common approaches. In addition, measurements can be evaluated by the software retrospectively without the requirement to repeat measurements. Sample preparation is suitable for a variety of food matrices and allergens, simplifying the required methods in lab.

P4

## Food hypersensitivity research programme at the Food Standards Agency

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The Food Standards Agency (FSA) has invested more than £20 million in over 60 different projects on food hypersensitivity, as part of its research programme. This research continues to have significant impact on our understanding of food hypersensitivity and has provided the FSA with opportunities to change policy in turn addressing consumer needs. Here, we present some recent FSA-funded projects which help inform policy so that food businesses can implement best practice in terms of allergen management and information provision, and to help people with a food hypersensitivity make safe and informed food choices when shopping and eating out of the home.

P5

## Neglected wheat species as a source of hypoallergenic lines: Unlocking the potential through genetic exploration

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Although the genus *Triticum* comprises a large genetic diversity, species and subspecies other than durum and common wheat are rarely used in production, consumption, breeding, and research. However, some neglected wheat species such as einkorn and emmer have been shown to be less immunoreactive compared to other wheat species. In the context of wheat allergies, amylase-trypsin inhibitors (ATIs), a group of non-gluten wheat proteins, play an important role in causing immune responses associated with baker's asthma and wheat sensitivity [1-3]. As little is known about the extent of variability in ATI levels and bioactivities within the broad *Triticum* spectrum, this study aimed to determine ATI levels, isomeric distribution, and functional properties in a broad collection of underutilized wheat lines with rare genomic constitutions to identify low-ATI lines for sensitive patients. The results confirmed the high ATI variability depending on the ploidy level and genomic constitution of the genotype. While diploid genotypes exhibited the highest trypsin inhibitory activity due to the presence of a specific einkorn trypsin inhibitor (ETI), significantly lower activities were observed in tetraploid and hexaploid cultivars. In contrast, α-amylase inhibitory activity followed an opposite trend and showed the highest values in hexaploid wheat, while no α-amylase inhibitory activity was detected in diploid genotypes. LC-MS/MS results revealed that the occurrence of ATIs was strongly associated with the presence of B and G genomes, while they were mostly absent in species containing only the A genome. Understanding the genetic effects on ATIs may help identify and develop low-ATI wheat lines, which is a promising strategy to combat diseases associated with grain consumption.

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**References**

1. Zevallos, V.F. *et al.*, 2017. Gastroenterology 152:1100-1113.
2. Junker, Y. *et al.*, 2012. Journal of Experimental Medicine 209: 2395-2408.
3. Geisslitz, S. *et al.*, 2021. Frontiers in Nutrition 8: 667370.

P6

**Effect of thermal processing and *in vitro* digestibility on the IgE-binding capacity of mollusc allergens**

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Molluscs are among the most widely consumed seafood species due to their nutritional properties. However, molluscs are also important allergenic foods, which are known to elicit severe/life-threatening allergic reactions in shellfish-sensitized/allergic individuals. Mollusc allergens comprehend distinct protein families, such as tropomyosins (panallergens), arginine kinases, paramyosins, among others [1]. In this sense, this work aims to evaluate the effect of food processing and *in vitro* digestibility on the IgE-binding capacity of shellfish allergens using sera from mollusc-allergic patients. For this purpose, distinct mollusc species (e.g., octopus, squid, clam) were submitted to different thermal treatments (boiling and oven-cooking) during different periods. Protein was further extracted, quantified by BCA, analysed by SDS-PAGE in non-denaturing conditions and immunoblotting with sera from mollusc-allergic patients. Some species were also digested following the INFOGEST 2.0 protocol [2]. SDS-PAGE results demonstrated a higher number of bands in processed than in the corresponding raw samples, indicating protein fragmentation triggered by thermal processing. Immunoblotting showed that, in most cases, the IgE-binding capacity of allergens like tropomyosins, paramyosins and arginine kinase is stronger in processed (boiled, oven-cooked) than in raw molluscs. Interestingly, water from boiling molluscs presented high quantity of proteins with IgE-binding capacity, meaning that part of the allergens is water-soluble and can be eliminated by discarding the boiling water. Still, the correspondent boiled sample contained a high amount of reactive protein(s). Most allergens seem to preserve their IgE-binding capacity during gastric digestion, though they were greatly reduced by subsequent intestinal digestion. These findings suggest that thermal processing might contribute to increase the allergenicity of molluscs, while gastrointestinal digestion can mitigate it. This is the first report on the evaluation of multiple mollusc species and the effect of single and combined food processing strategies on mollusc IgE-binding capacity.

**Acknowledgments**

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**References**

1. Fernandes, T.J.R. *et al.*, 2015. Food and Agricultural Immunology 26: 848-869.
2. Brodkorb, A. *et al.*, Nature Protocols 14: 991-1014.

P7

**PCR- based detection of allergenic oil crops in foods**

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Oleaginous crops, such as soybean, maize, and sunflower, are widely used in food production however they are recognized as important food allergens. Accurate detection of oil crops is required for food authentication, quality and safety assessment, proper labelling, consumer information and health protection. This study presents new polymerase chain reaction (PCR) methods for reliable detection of allergenic ingredients in food. The conducted research included several steps, such as the identification of species-specific genes and DNA sequences by *in silico* genome data analysis; design of oligonucleotide primers using bioinformatics tools; genomic DNA extraction; DNA quantification by spectrophotometer; development and optimization of uniplex and multiplex PCR systems; evaluation of genomic DNAs and PCR products by agarose gel electrophoresis. Seeds, flours, and various processed food products were investigated, including cold-pressed and refined cooking oils. Various DNA isolation approaches and several PCR protocols were applied. A comparison of the obtained results revealed the best DNA extraction and PCR amplification methods for each studied foodstuff. New efficient DNA markers for soybean lectin gene, maize zein and invertase gene, as well as sunflower helianthinin gene were identified by uniplex PCR methods. Triplex PCR was developed for simultaneous identification of soybean, maize, and sunflower. Testing of various food products demonstrated that the new PCR methods developed in this study are useful for the reliable detection of oily ingredients in food.

**Acknowledgments**
This work was supported by Shota Rustaveli National Science Foundation of Georgia (SRNSFG) (Grant AR-22- 636).

P8

## Tropomyosin variants from *Hermetia illucens* (black soldier fly): identification, characterization and allergenicity

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The constant growth of world's population has led to search new food sources. According to the U.N. Food and Agriculture Organization (FAO), insects have a promising potential as an alternative food source, with both environmental and health benefits. In parallel with development of regulations around the use of insects for human consumption, the associated food allergy risks should be explored. Allergic reactions to edible insects have been reported as well as the possibility of cross-reactivity with crustaceans and house dust mites. Tropomyosin (TPM) and arginine kinase are the primary allergenic proteins in crustaceans and house dust mites. *Hermetia illucens*, black soldier fly (BSF) is one of the most interesting insects for the potential use in human diet and, in this perspective, it is essential to assess possible cross-reactivity with known Arthropoda allergens. In this work, we investigate the allergenic potential of two variants of TPM identified in BSF genome by bioinformatic analysis. TPM isoforms were expressed as recombinant proteins in *E. coli*. Purified TPM variants, characterized by size exclusion chromatography, cross-linking assays CD spectroscopy and LC-Mass spectrometry showed alpha-helices secondary structures assembled to form tetramer complexes. Protein stability to increasing temperature up to 55°C and to different pH (in the range 3-9) was also investigated by SDS-PAGE and CD spectroscopy showing that change in pH value has little effect on secondary structure of both proteins, which is instead affected by the increase of temperature. The stability of the allergens to the digestion process was tested by performing an *in vitro* digestion assay. Peptide released were identified by high resolution mass spectrometry, in order to check if the predicted epitope regions are kept unchanged. The immunoreactivity of the two TPM isoforms were tested by performing IgE-immunoblotting assays on sera from patients with food allergy to shrimps and/or airway allergy to mites. Interestingly, both the recombinant proteins were recognized by IgE of allergic patients pointing out a cross-reactivity of these *H. illucens* TPM variants with shrimps and mites. In order to protect potential allergic consumers, these findings highlight the importance of a complete molecular characterization of the different allergens, and of the thorough assessment of possible cross-reactivities.

**Acknowledgements**
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P9

**Evaluation of gluten recovery from two sources (barley and rye flours) into different matrices using GlutenTox® ELISA Rapid G12 and two other commercial ELISA kits**

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Claimed gluten sources for most of the commercial gluten ELISA kits used by the food industry or by independent third-party laboratories are wheat, barley, and rye. However, the gluten content of barley and rye are overestimated in some of these kits, which have been able to be accredited under the ISO 17025 quality standard by many laboratories. These accredited methods (under the scope of 'gluten in foods') can produce inconsistent quantification of gluten residues in foods containing gluten from barley and rye. The GlutenTox® ELISA Rapid G12 is the first assay to be granted AOAC RI PTM certification using wheat, barley, and rye flour. The purpose of this study was to evaluate gluten quantification and recovery from barley and rye flours individually spiked into different matrices using the GlutenTox ELISA Rapid G12 Kit (KIT3075) and two other commercial kits, RIDASCREEN© Gliadin (R7001) and RIDASCREEN Total Gluten (R7041). The study was conducted using 4 food matrixes (gluten-free soy flour, maize bread, seasoning mix, and rolled oats) artificially contaminated with gluten from barley or rye flour at different concentrations: 0, 10 and 20 mg/kg. For each matrix and gluten contamination level, 5 individually extracted test portions were analysed. Recoveries obtained for gluten concentration from barley and rye flours were: 291-671% for Ridascreen Gliadin Kit (R7001); 101-149% for Ridascreen Total Gluten Kit (R7041) showing a slight elevation in some rye-contaminated samples, recoveries between 159-167%; and 80-143% for GlutenTox ELISA Rapid G12 or slight elevation, recoveries from 153-192% (only one sample (maize bread) contaminated with rye flour at 20 ppm of gluten showed moderate overestimation, recovery 277%). In conclusion, this study confirmed the great overestimation of gluten content when using the Ridascreen Gliadin Kit (R7001) on samples spiked with barley and rye flours. In this sense, ISO 17025-accredited laboratories with R5-sandwich-ELISA (Méndez method), Ridascreen Gliadin Kit (R7001) could be using a non-fit-for-purpose kit, considered a reference method, to quantify gluten in foods from barley and rye. Ridascreen Total Gluten Kit showed a gluten quantification from barley and rye flours more accurate compared to the Ridascreen Gliadin Kit. GlutenTox ELISA Rapid G12 Kit yielded results comparable to those obtained with the Ridascreen Total Gluten Kit in most of the cases or showed a slight overestimation; therefore, it can also be used to quantify gluten from barley and rye.

P10

**Development of an all-in-one ready-to-use real time PCR-based system for the simultaneous detection of six insect species in food**

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Within the EU market, insect-based products are certainly one of the prominent examples of novel foods. Nonetheless, insects are known for their ability to trigger allergy, and to contain some pan-allergens common to other invertebrates, such as crustaceans, molluscs, and nematodes, thus posing a potential threat especially to allergic individuals sensitized to crustaceans and to house dust mites. Moreover, insects as food are able to primarily sensitize new individuals. Insects can bring new food allergens to the market; therefore, analytical methods of detection are urgently required. According to the Regulation 2015/2283, novel foods must undergo a risk assessment before being allowed for human consumption. The EC has so far authorized the marketing of six food products from four of the main species of insect currently farmed: the yellow mealworm (*Tenebrio molitor*), the migratory locust (*Locusta migratoria*), the house cricket (*Acheta domesticus*) and the buffalo worm (*Alphitobius diaperinus*). Moreover, an application for *Hermetia illucens* meal (black soldier fly) is currently under risk assessment at EFSA, while the only application for the banded cricket *Gryllodes sigillatus* that reached EFSA was withdrawn by the applicant last year, despite this species is already authorized as feed for aquaculture, pigs, and poultry. The aim of this study was to develop a pre-spotted ready-to-use real time PCR system to simultaneously detect six species of commercially available insects in food, and it was carried out in the frame of the ALLERGEN-PRO project, financed by the German Federal Ministry of Food and Agriculture (BMEL). All the PCR systems employed were either in house developed (*A. diaperinus, A. domesticus*, *G. sigillatus, H. illucens*), or adapted from the existing literature (*L. migratoria, T. molitor*). Each system has been individually in-house validated on both commercial and processed model foods, to establish their applicability and sensitivity in real foods containing trace levels of insect. Real time PCR 96-well plates were pre-spotted with primers and probes (plus the DNAs of the positive controls) of eight different systems: in addition to the species-specific systems, two additional amplification and inhibition control systems have been added. The plate layout is simple and intuitive, and the presence of internal controls ensures that five samples can be tested in duplicate without the need of pre-screening tests, adding only the mastermix. These all-in-one ready-to-use plates can be easily stored by laboratories performing routine checks and used to rule out the presence of potentially dangerous insects for allergic consumers.

P11

**Automated and sensitive allergen detection with a portable microfluidic platform integrating sample preparation and a smart immunoassay**

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Food allergy is major global issue, and children, in particular, are being affected in increasing numbers. Because there is no cure yet, the only solution for allergic patients is strict avoidance of exposure to food allergens. In response, European legislation (Regulation (EU) 1169/2011) dictated the mandatory labelling of 14 potentially allergenic ingredients. Food industry therefore requires easy-to-use analytical tools to detect the presence of unwanted allergens. Portable diagnostic systems are gaining attention in healthcare, environmental monitoring, and agro-food sectors. Bypassing traditional laboratories with rapid and on-site analysis enables time and money saving. By using centrifugal microfluidics, neither pumps nor tubings are needed to perform precise and accurate liquid handling steps creating an easy to use and portable platform. In this context, we aim to demonstrate that the integration of portable sample preparation strategies and immunological assays leads to innovative technological solutions able to lower on-site detection limits. Soybean, one of the most common sources of dietary protein and a major food allergen, was selected as a model for the development of a prototype centrifugal microfluidic cartridge. A denaturing extraction protocol was developed and optimized for complex and processed matrices. The different steps of the sample preparation and analysis were integrated in the injection moulded cartridge, including the clean-up of the denatured extracted sample, the capture of the analyte on fluorescent microbeads functionalized with specific antibodies and the lateral flow immunochromatographic assay. Particular attention was paid to the functionalization of the fluorescent beads with in-house developed anti-soybean antibodies. Several regioselective modification strategies were considered to prevent bead aggregation due to cross-linking and interferences with the antigen-binding regions. These optimizations increased the immunoreactive fraction and therefore improved the overall detection sensitivity and reduced biologicals consumption.

P12

**Impact of microfluidization, enzymatic treatment and their combination on soybean allergens**

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Food allergies are a growing global health problem, with soy proteins being a significant allergen due to its widespread use in various food products. Several researchers have tried different methods to reduce soybean allergens to solve its challenges to human health. In our work, research on microfluidization, enzymatic, and combination of enzymatic with microfluidization was investigated on soybean protein isolate. The innovative technique, microfluidization, has been tested for other food allergens, such as peanut or milk, but studies on soybean allergens still need to be done. This method was chosen to understand the effect of high shear stress and high-velocity impact, which generally occurs from the walls and the fluid itself during microfluidization. During our research, the allergenicity of soybean protein isolates was evaluated by indirect ELISA and western blotting using human sera of patients who reported soybean allergy. Microfluidization alone has proved ineffective in reducing the allergenicity of soybean protein isolate, even increasing it under specific treatment conditions. Moreover, the enzymatic hydrolysis results demonstrated almost complete hydrolysis of all the major soybean allergens. Combined with enzymatic treatments, it did not significantly enhance the obtained allergenicity reduction and degree of hydrolysis.

P13

## Allergy to goat milk and the possible role of goat milk in non-IgE mediated allergy or milk allergy prevention

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Goat milk has been an important part of human nutrition for millennia. Allergy to goat milk, not associated with cow's milk allergy, is a rare disorder and only a few case reports of a single goat milk allergy have been described in the literature. Goat milk proteins have a large homology with cow's milk proteins and show cross-reactivities in IgE-mediated allergy and should therefore not be advised for infants with cow's milk allergy. However, there are indications that goat milk could be beneficial in some types of non-IgE-mediated allergy or even for prevention of sensitization to milk proteins, but controlled clinical studies are needed to confirm hypothesis. Although goat milk and cow's milk proteins show a large homology, the composition of the proteins is different. For example, goat milk has lower alpha-s1-caseins and higher beta-casein levels, making goat milk less allergenic. Another advantage is that goat milk has been associated with faster and more efficient digestibility than cow's milk proteins. This could lead to the production of tolerance inducing peptides (tolerogenic peptides) during early digestion compared to cow's milk, known to produce allergenic peptides during digestion. Thus, goat milk might contribute to a better oral tolerance induction to milk proteins. Therefore, due to its lower allergenicity and easier digestion, goat milk-based formula could be a better choice over cow's milk-based formula as the first source of protein after a breast-feeding period.

P14

## Identification of effective DNA markers for wheat glutenin

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Wheat (*Triticum aestivum)* belongs to potent allergens and causes significant health disorders. To prevent the disease, sensitive individuals should not use the allergenic product. Wheat allergenicity is primarily defined by glutenin proteins which are also closely related to the baking quality of wheat flour, as well as the quality of pasta and other processed products made from wheat. Therefore, the reliable identification of glutenin in food products is essential both for food safety assessment and health protection, as well as for efficient food manufacturing technology. The aim of this study was to discover new efficient DNA markers for the accurate identification of glutenin in processed products. For this purpose, several sets of oligonucleotide primers targeting the *Triticum aestivum* low molecular weight glutenin subunit gene were designed using bioinformatics tools. Wheat grains, flour and various processed products were investigated. Foodstuffs were purchased from supermarkets and also prepared in the laboratory under different conditions. DNeasy plant mini kit (Qiagen) was applied for genomic DNA extraction. A spectrophotometer and agarose gel electrophoresis were used to assess DNA quantity, purity, and integrity. A conventional polymerase chain reaction (PCR) method was used for DNA amplification. PCR products were evaluated by agarose gel electrophoresis. After optimization of PCR conditions using wheat flour DNA template, new markers for low molecular weight glutenin subunit gene were identified, i.e., 83 bp, 93 bp, 99 bp, 109 bp and 259 bp fragments of this gene. Examination of other plant species confirmed the high specificity of the 83 bp marker for wheat glutenin. Testing of various processed products identified the 83 bp and 259 bp amplicons of the glutenin gene as the most effective markers for glutenin detection in highly processed foods. The obtained results indicate that the PCR-based DNA markers described in this study can be successfully used to monitor wheat glutenin in foods.

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P15

## Reducing allergenicity of infant formula by novel processing technologies

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Infant formulas containing cow's milk are widely used in infant nutrition, but some infants cannot tolerate them due to known allergens. The most common food allergy in young children is cow's milk protein allergy (CMPA). The allergy is classified as either an IgE-mediated or non-IgE-mediated allergy, and it can cause anaphylaxis reactions in severe cases. Cow's milk consists of approximately 30--35 g/l proteins, containing over 25 different proteins, but only a few are allergenic. The most prominent cow milk proteins involved in allergic reactions in children are ALA, BLG, and αs1-CN. Since bovine milk is a complete food with high nutritional value for children, excluding it from their diet increases nutritional risk. Therefore, it is possible to reduce allergy risks for children by using cow milk substitutes such as partially, extensively, and fully hydrolysed formulas (e.g., enzymatic hydrolysis or heating and ultrafiltration), plant-based formulas (soy and rice) as well as other mammalian milk types, or by using alternative technological methods (e.g., fermentation, and novel non-thermal). Although heat treatment and enzyme hydrolysis have been used to produce hypoallergenic infant formulas, modulating allergenic epitopes depends on the amount of heat treatment applied, which may also lower their nutritional value. Furthermore, enzymatic hydrolysis may not target allergenic epitopes, and allergenicity may persist. Also, the released peptides may affect the taste and functional properties of the final products. Thermal and non-thermal methods modify allergen reactivity by changing protein structure and altering IgE binding sites. However, it is equally critical to maintain food origin and quality while reducing food allergenicity by altering protein. An appropriate approach would reduce milk protein allergenicity while maintaining its overall composition and sensory properties. Thus, some novel non-thermal techniques (e.g., high pressure, microwave, ultrasound, pulsed light, cold plasma, pulsed electric field) have been explored for the production of hypoallergenic foods, with minimal negative effects compared to substantial changes caused by thermal treatment. Accordingly, these treatment results can potentially modify cow proteins to alter their allergenicity and enhance infant formula safety for cow milk-allergic babies.

P16

**Implementation of high-pressure processing in inactivation of the allergens from apple and celery juices**

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Food allergy is an abnormal immunological response or allergic reaction when certain foods are consumed. It is considered a significant public health concern, affecting 1-2% of adults and 3-6% of children living in developed countries. Celery is a valuable dietary vegetable containing many different vitamins, minerals, and polyphenols. It is a source of dietary fibre and potassium and is thought to have anti-inflammatory benefits. However, celery allergy was observed in 6.3% of the general population worldwide. Apples (*Malus domestica* Borkh.) are among the most consumed fruits globally offering nutrients and nutraceuticals like polyphenols and other phytochemical. Although sensitization to apples is associated with the cross-reactive birch pollen aeroallergen due to the structural homology of allergenic proteins. High-pressure processing (HPP) is a novel, nonthermal processing technology already used worldwide commercially. The possible elimination of food allergen by applying HPP were measured by ELISA technique. In this study, samples of each crop were prepared as juice and were subjected different treatments (200, 300, 400, 500, and 600 Mpa for 1, 3, 5, 7, and 9 min). According to results, applications of HPP did not influence the concentrations of allergens in celery and apple juices. Nevertheless, further investigation in applicability of this emerging technology in reducing of allergens in similar food products is recommended.

P17

## Molecular dynamics simulation of allergens in food: An *in silico* approach

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Food processing techniques have evolved to improve food products' safety, shelf life, convenience, and nutritional and functional properties. With the emergence of novel processing methods, it is crucial to understand the impact of external stressors on the biomolecules present in food products, such as carbohydrates, proteins, and lipids. Food process engineering aims to understand better and manipulate food processes to produce a desirable finished product that meets the population's needs. Simulation modelling is an essential tool in achieving this goal, as it combines physics, statistics, applied mathematics, and computing to accurately predict the result of a given processing treatment. Atomistic molecular dynamics (MD) simulation is a computational methodology that models molecular systems based on Newton's equation of motion. MD simulations have been used to study the effects of food processing on biomolecules, identify potential modifications that may reduce allergenicity while preserving functionality, and improve experimental design efficiency. While MD simulations have proven to be a valuable tool in food processing research, there is still much work to be done in this field. In the first case, MD simulations have been used to investigate the structural changes in proteins that can reduce their allergenicity. By studying the protein's conformational state, researchers can identify potential modifications preventing the protein from triggering an allergic reaction. For instance, simulations of Ara h 6, a major allergen in peanuts, revealed that reducing the surface area of the protein could limit its interaction with immune cells and thus reduce its allergenicity. In the second case, MD simulations have been used to study the effects of food processing on protein structure and allergenicity. Thermal and electric treatments are common methods used to modify the properties of food proteins, but they can also affect their allergenicity. By simulating these processes at the molecular level, researchers can identify the optimal conditions for reducing allergenicity while preserving the desired functional properties of the protein. MD simulations have become an increasingly popular tool in food allergy research due to their ability to provide detailed insights into the molecular mechanisms underlying allergenicity. By simulating the behaviour of proteins at the atomic level, researchers can identify potential modifications that may reduce their allergenicity without compromising their functionality. This approach has yielded promising results in several food allergens, including peanuts, soybeans, cow's milk, hazelnuts, kiwifruit, and eggs. Overall, MD simulations are essential in developing new strategies for reducing food allergenicity. By providing detailed insights into the molecular mechanisms underlying allergenicity, these simulations can help identify potential modifications that may reduce the risk of allergic reactions while preserving the desired functional properties of the protein. While there is still much work to be done in this field, MD simulations have proven to be a valuable tool in the fight against food allergies.

P18

## The matrix effect on allergenicity potential

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The food matrix is a complex system encompassing all constituent elements in food production. Food allergens, in general, are glycoproteins ranging from 10 to 70 kDa, often present in processed foods and consumed as dietary components by the body, which possess multiple IgE binding epitopes. Their structural stability is influenced by the food matrix, which is mostly released from the food during food processing, oral processing, and digestion. The food matrix affects the digestion and allergenicity of food allergens in two ways. Firstly, it directly interacts with the allergen, influencing its gastrointestinal digestibility, mucosal absorption kinetics, and performance in the immune system, which in turn affects the allergenicity of the allergen. Secondly, the food matrix can also act on the digestive environment and influence the digestion and allergenicity of allergens. Therefore, the composition of the food matrix, the degree of processing and digestion of its components, and interactions with allergens impact how well they digest in the GI tract, transport through the epithelium, and their capacity to sensitize and trigger allergic responses. Before being consumed, most foods undergo various processing steps to enhance their sensory qualities, increase their nutritional value, prolong their shelf lives, and stabilize them. Furthermore, various food processing techniques have been linked to changes in allergens, according to several studies. The antigenic properties of allergens could change as a result of various processing procedures (such as heat treatment, represented by cooking and baking, and nonthermal treatment, represented by high pressure and ultrasound), which could alter the physicochemical characteristics and the three-dimensional structure of proteins and subsequently the IgE binding epitopes. Depending on the processing technique, the kind, and degree of structural alterations could vary and influence food allergens' digestibility and allergenic properties. This is particularly true for conformational epitopes, which rely on tertiary structure.

P19

## Tracing yellow mealworm (*Tenebrio molitor*) flour as a novel allergenic ingredient in processed foods

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Edible insects are considered a valuable source of nutrients such as polyunsaturated fatty acids, essential amino acids, micronutrients, and proteins, having a nutritional value comparable or superior to those of both chicken and beef. Moreover, they present a lower negative environmental impact than conventional animal-derived protein sources, with less greenhouse gas emissions and water pollution and higher feed conversion efficiency. The yellow mealworm is the larval form of the insect species *Tenebrio molitor*, being the first insect-derived food product with completed evaluation for human intake. However, it might induce allergic reactions to new IgE-binding proteins in crustacean-allergic and/or dust mite-allergic subjects (pan-allergens) [1,2]. Therefore, its detection at trace levels in foods is a key issue to verify labelling compliance and protect sensitized individuals. This work aimed at developing a new real-time PCR approach for the quantitative detection of *T. molitor* as a potential allergenic food. For this purpose, reference mixtures simulating the production of pork sausages and wheat biscuits, containing known amounts of insect flour were used. Real-time PCR with a TaqMan probe targeting the cytochrome b gene of *T. molitor* allowed detecting down to 2 fg of insect DNA, and 1.0 and 0.1 mg/kg of mealworm flour in autoclaved sausages and baked biscuits, respectively [3]. Generally, the method revealed acceptable analytical performance parameters. Food matrix and processing significantly affected real-time PCR data, highlighting the importance of using appropriate calibration models for quantitative analysis. Validation results using blind mixtures of both food matrices showed that most precision and trueness data were within the acceptance criteria. Application results indicated that the analysed samples of chocolates and protein bars were complying with the labelled information on the mealworm detection(quantification. In the present work, a novel highly sensitive and specific method was successfully proposed as a reliable tool to quantify yellow mealworm in processed foods at trace levels.

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**References**

1. Turck, D. *et al.*, 2021. EFSA Journal 19: 6343.
2. Pali-Schöll, I. *et al.*, 2019. Trends in Food Science & Technology 84: 45-48.
3. Villa, C. *et al.*, 2023. Nutrients 15: 482.

P20

## New automated immunoanalysis method based on nanoparticles for the gluten quantification in food

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Celiac Disease (CD) is one of the most common food intolerances which can affect up to 1% of the population. It comes along with serious damage of the mucosa in the small intestine and is caused by the storage proteins – called 'gluten' – of wheat, barley, and rye. Sensitive individuals need to stick to a strict gluten-free diet so, the correct quantification and labeling of foods are crucial for following a gluten-free diet. There are different analysis methods commercially available to determine the presence of gluten, being the lateral-flow assays and ELISA the most used. Both are based on the antigen-antibody reaction. This work presents a new method for the quantification of gluten, based on an automated turbidimetric immunoassay in a spectrophotometric analyzer. In this method, the antibody is immobilized on the surface of latex nanoparticles, which aggregate in the presence of gluten. This turbidity is measured photometrically, and the absorbance obtained is proportional to the gluten concentration in the sample. For this purpose, a highly specific monoclonal antibody that recognizes the 33-mer fragment of gluten has been developed. This fragment is known for its high immunogenicity and toxicity in celiac patients. Validation data on the BioSystems Y15 analyzer indicate that the linear range of the method is from 0 to 40 mg/kg, with a correlation coefficient ($r^2$) greater than 0.99 with a limit of quantification (LOQ) of 5 mg/kg. Recoveries obtained for different concentrations of purified gliadin from the Prolamin Working Group or with wheat flour are in the range of 90-110% in all cases. In addition, comparisons made in against the reference method for gluten (based on the R5 antibody, AOAC-2012.01) demonstrate good correlation. The Gluten Y15 BioSystems together with the developed extraction solution is a useful tool for automated, fast, precise, and simple determination of gluten. Automation of the measurement in the BioSystems Y15 analyzer improves the precision and accuracy of the results, eliminating possible measurement errors by the user, and allows for great flexibility in the analysis of samples, from a few to high throughput of 75 samples per hour.

P21

**Developing an ip animal model to predict sensitising capacity of novel food**

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It is widely accepted that the global population is increasing, with an estimated rise to 9.7 billion by 2050. Thus, there is a great focus on developing sustainable novel food to cater for the growing demand of food protein. Yet, the introduction of novel foods into the food supply chain and their subsequent consumption may pose a potential health risk to humans, including the potential emergence of new food allergies. However, at the moment there are no tools available for predicting the sensitising capacity of new food proteins. As food allergy is a complex disease and as sensitisation cannot be studied in humans, animal models may provide a good option for such predictive model. However, to develop an animal model with predictive capacity, a first step would be to show that the animals would react and be able to rank foods according to their allergenicity in the same way as humans. We here present a first attempt to develop a predictive ip animal model for studying the sensitising capacity of novel food, using the low allergenic gelatine, spinach, and beetroot protein extracts, the medium allergenic pea protein extract, and the high allergenic hazelnut and peanut protein extracts.

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P22

**Measures to manage allergenic risks with edible insects**

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From October 2020, certain species of insects can be marketed as food within the European Union although they are not yet authorized novel foods. Therefore, the Swedish Food Agency (SFA) initiated a risk management process about allergic reactions to edible insects and possible measures for stakeholders. The permission is due during a transitional period. Currently, four insect species are authorized novel foods. The concept of risk management at the Authority level was followed. This is defined in article 3.12 in 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”. The following can be conluded from the risk assessment. Shellfish allergy is one of the most common allergies in the adult population globally. There is a risk for cross reactivity to insects among shellfish allergic individuals, both theoretically and clinically. Certain protein similarity exists between species within the phylum *Arthropoda* to which both insects and shellfish (crustaceans) belong. Although there is a need to further study the allergic cross-reactivity, the research shows that the probability of clinical cross-reactions is relatively high, up to 80 percent. This cross reactivity can be severe with symptoms such as asthma and anaphylaxis. Other legitimate factors considered, are: (i) the health care system gives patients with food allergy individual advice as part of their dietary treatment; (ii) insects have previously not been a substantial part of the diet within the EU and the knowledge about these allergic cross-reactions is probably limited among health care professionals and allergic consumers; and (iii) the measures should be proportional to the risk and should not be more extensive than measures taken to decrease the risk to develop primary allergy or unexpected allergic reactions to food allergens that constitute a higher risk, for example milk or peanuts. The SFA assessed that the following voluntary information regarding the cross reactivity would be beneficial during the transitional period: (i) information to health care professionals and consumers regarding the risk of cross reactivity; and (ii) guidance to Swedish food business operators to give clear information on the identity of the insect species and voluntary information on risk for allergy in connection with marketing of insect food products, advising to use the sentence “Insects may cause allergic reactions in people with allergy to shellfish or house dust mite”.

P23

**Expanding the scope of a routine LC-MS/MS approach to allergens testing**

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Food allergies are the leading cause of anaphylaxis, an acute, potentially deadly allergic reaction. The prevalence and severity of food allergies are rising, with approximately 150 million people suffering from food allergies worldwide. Presently, there is no cure for food allergies, and sufferers must rely on the correct labelling of foods to avoid consuming allergens. Hence, developing sensitive and accurate analytical methods to screen for the presence of allergens in food products is necessary to prevent potentially life-threatening health problems for allergy sufferers. SCIEX vMethod application for food allergen testing previously provided a workflow for sample preparation and LC-MS/MS detection of 13 distinct allergens, including egg, milk, almond, Brazil nut, cashew, hazelnut, pine nut, pistachio, pecan, walnut, peanut, soy and sesame. In addition, a highly selective and sensitive LC-MS/MS method was developed to quantitate wheat gluten proteins and to identify what are the major grains in the food matrix at the same time by monitoring ray, barley, and oats unique peptides in the bakery, fermented beverage and baby formula products.

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**IgG-binding capacity of γ-conglutin, a lupine major allergen, as affected by thermal processing and gastrointestinal digestion**

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Lupine has been increasingly used as a functional food and as an ingredient in all kinds of food products (bakery, confectionary, snacks) due to its high nutritional value, protein content and technological properties. However, allergy to lupine has been reported with an increasing prevalence in the last years, causing serious adverse immunological reactions in sensitised/allergic individuals [1]. Gamma-conglutin is one of the most relevant lupine proteins and it is considered a major allergen since allergic patients presented an IgE-binding frequency higher than 50% [2]. Therefore, this work aimed at characterising gamma-conglutin from the most economically important lupine species (*Lupinus albus, L. luteus* and *L. angustifolius*) and assessing the effect of thermal processing on their immunoreactivity. Following the application of food processing, the effect of simulated gastrointestinal (GI) digestion was also evaluated. For this purpose, three model foods of wheat pasta were prepared containing 35% of lupine flour from the three most common lupine species. Then, model samples were submitted to a boiling process for 5 min to simulate pasta preparation. After extraction, the protein profile of different model samples was evaluated by SDS-PAGE in non-reducing conditions. IgG-binding capacity of γ-conglutin was assessed by immunoblotting with specific antibodies. The harmonised GI digestion protocol from INFOGEST [3] was then applied to model pasta for digestibility studies. SDS-PAGE results suggest that the protein profile of lupine seeds is very different among species/varieties. A distinct IgG-binding pattern was also observed for γ-conglutin, depending on the lupine species. The boiling treatment seems to induce a reduction in γ-conglutin immunoreactivity due to an increased modification of protein integrity. Simulated digestion led to an even more extensive damage of the protein structure, reducing IgG-binding to γ-conglutin and the potential subsequent presentation to immunocompetent cells. This reduction was more significant in the intestinal phase, leading to the complete disappearance of IgG-binding capacity. These results provide a better insight into the impact of thermal processing and GI digestion on the immunoreactivity of γ-conglutin and, consequently, on its potential allergenicity, allowing to provide a useful contribution to food industry towards the development of products with reduced allergenicity.

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**References**

1. Villa, C. *et al.*, 2020. Comprehensive Reviews in Food Science and Food Safety 19: 3886-3915.
2. Magni, C. *et al.*, 2005. Journal of Agricultural and Food Chemistry 53: 4567-4571.
3. Brodkorb, A. *et al.*, 2019. Nature Protocols 14: 991-1014.

P25

**Comparative study of multiple celery DNA kits in different food matrices**

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Worldwide approximately 3% of adults and 6% of children suffer from food allergies for who safe production and correct labelling of food products are of paramount importance. However, allergen risk assessment is complex as very low levels of unintended presence in food products already can evoke a harmful physiological response in allergic consumers. Food industry is increasingly adopting self-control test methods to analyse food safety parameters in the trade or production chain, allowing them to respond quickly and avoid potential safety or quality risks. However, there are questions on the quality performance of fast self-control detection methods, especially when used with complex matrices or processed foods. In addition, when precautionary allergen labelling will be implemented based on references doses, sensitive and reliable fast allergen detection methods are required. We present the results of a comparative assessment of four commercially available test kits for the DNA detection of celery in food products. Five product groups representing different sectors of the AOAC food-matrix triangle were identified to potentially contain celery. From each group blank and incurred (labelled to contain celery) food products were selected, of which the blank food products were in addition spiked with 1, 3 and 10 ppm protein celery per kg food product. Prior to qPCR analysis DNA was extracted using the manufacturers' recommended DNA extraction method, and DNA and qPCR analyses were performed exactly according to the manuals in order to comparatively assess the quality performance of the kits. Results show that three commercially available test kits were able to detect down to 1 ppm in four spiked product groups. The fourth test kit, specified with an LOD of 25 ppm, could not detect the spiked materials. In three of the five product groups incurred samples were shown to contain celery with three kits, samples from two challenging product matrix? groups showed inconclusive results, confirming the influence of complex matrices on the detection ability of the kits. In addition, quantification of celery in the different food products resulted in variable quantities between the different kits. Although trends were observed, no conclusive quantity could be assigned to any product. In general, it can be concluded that the test kits qualitatively perform according to their specifications, however some specific difficult matrixes gave inconclusive results. Furthermore, quantification is challenging for all kits in all food product groups.

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## Characterization of mono- and polyclonal antibodies for the detection of soy in foods

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Soybean (*Glycine max*) is the most used vegetarian protein source and a common ingredient of industrially produced foods. In addition to several nutritional and health benefits, soybean is classified as an allergenic source affecting 0.2-0.4% allergic individuals worldwide, even causing severe allergic reactions such as anaphylaxis. Therefore, the detection of soybean allergens in food is a crucial step in allergen avoidance and contributes to allergy risk management. Soybean specific monoclonal antibodies were produced by immunizing Balb/c mice with defined soybean extracts containing high amounts of the respective soybean allergen. Additionally, soybean-specific polyclonal antibodies were collected from the serum of soybean immunized rabbits. The antibodies were characterized with different immunological methods including Western Blot and different formats of ELISA. Cross-reactivity tests were performed with extracts of peanut, lentil, bean, hazelnut wheat, lupine and milk among others to exclude false-positive results and to confirm the specificity of the soybean-specific antibodies. Further, soybean containing and soy-free foods were analysed. Four monoclonal antibodies were obtained after successful immunization of Balb/c mice with soybean extracts and the following cell fusion and cell cultivation. Western Blotting and ELISA using well-characterized soybean extracts confirmed the specificity of the monoclonal antibody to soybean allergens. As expected, the polyclonal soybean-specific antibodies recognized several soybean allergens and other allergens of cross-reactive extracts. The applied soybean-specific antibodies recognized soybean allergens in different soybean extracts and soybean-containing foods. In conclusion, antibody-based immunoassays are a suitable, state-of-the-art method, to specifically detect food allergens in different extracts and food samples. These soybean-specific antibodies will be further used in the ELISA and LFD development to improve allergen screening of food material.

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**Mustard seed major allergen Sin a1 activates intestinal epithelial cells and dendritic cells, driving type 2 immune responses**

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Mustard seed belongs to the food category of mandatory labelling due to the severe reactions it can trigger in allergic patients. However, the mechanisms underlying allergic sensitization to mustard seed are poorly understood. The aim of this work is to study sensitization to the mustard seed major allergen Sin a 1 via the intestinal mucosa, employing an *in vitro* model mimicking allergen sensitization through the intestinal barrier (IEC). Sin a 1 was isolated from total protein extract. Human HT-29 cell line was used as model for IEC. Dendritic cells (DC) were derived from monocytes from healthy donors, and naïve T and B cells and stem cell derived mast cells (MC) were obtained from additional healthy donors. A sequential *in vitro* system of consecutive (co-)cultures was employed to study the Sin a 1 sensitization capacity: IEC/DC (transwell), DC/T-cell, T/B-cell and MC. Immune profiles were determined by ELISA and flow cytometry. Sin a 1 drives type-2 cytokine secretion in IEC (CCL20, IL33), IEC/DC co-culture or DC alone (IL-25) and Sin a 1 primed IEC/DC or DC induced T-cell activation (IL-4). IgG secretion in the T-cell/B-cell phase was enhanced after the presence of Sin a 1 in the first stages of the co-culture. Anti-IgE activation promoted IL-13 release by MC primed with the supernatant from B-cells co-cultured with Sin a 1-IEC/DC or Sin a 1-DC primed T-cells. In conclusion, Sin a 1 enhanced the release of type-2 inflammatory mediators by epithelial and dendritic cells, instructing type-2 responses in T-cells that resulted in B-cell activation, and finally MC activation upon anti-IgE exposure. This indicates that via activation of IEC and/or DC, mustard seed allergen Sin a 1 is capable of driving type 2 immunity which may lead to allergic sensitization.

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## New novel food approvals in the European Union since 2018: How impactful are allergenicity risk assessments?

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With the global population estimated to reach 9.7 billion by 2050, the introduction of novel foods in the European Union (EU) will strongly contribute to the security and sustainability of Europe's food supply. This means that there is an increased demand for protein-rich novel food in the indefinite future. In the EU, novel foods are food products that have not been consumed to a significant degree by people in EU Member States before 15 May 1997, which include edible insects or food derived from newly developed production processes. For novel foods, appropriate safety assessments, including an allergenicity risk assessment, must be performed to ensure that they are safe for human consumption. However, the current methods for assessing the allergenicity of novel food proteins is far from complete. Since the introduction of EU Regulation 2015/2283 regarding novel food and its subsequent implementation from 1 January 2018, despite more novel food dossiers being submitted to the European Commission, allergenicity assessments of novel food cannot be conducted appropriately due to a lack of accurate and validated tools. This poster will provide insight into the trends, duration from application and approvals, and the type of allergenicity risk assessments performed in the approval of new novel food since 2018.

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P29

**An advanced *in vitro* human mucosal immune model to predict food sensitizing allergenicity risk: A proof of concept using ovalbumin as model allergen**

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The global demand of sustainable food sources leads to introduction of novel foods on the market, which may pose a risk of inducing allergic sensitization. Currently there are no validated *in vitro* assays mimicking the human mucosal immune system to study allergenicity risk of novel food proteins. The aim of this study was to introduce a series of sequential human epithelial and immune cell cocultures mimicking key immune events after exposure to the common food allergen ovalbumin from intestinal epithelial cell (IEC) activation up to mast cell degranulation. This *in vitro* human mucosal food sensitizing allergenicity model combines crosstalk between IEC and monocyte-derived dendritic cells (moDC), followed by coculture of the primed moDCs with allogenic naïve $CD4^+$ T cells. During subsequent coculture of primed $CD4^+$ T cells with naïve B cells, IgE isotype-switching was monitored, and supernatants were added to primary human mast cells to investigate degranulation upon IgE crosslinking. Mediator secretion and surface marker expression of immune cells were determined. Ovalbumin activates IEC and underlying moDCs, both resulting in downstream IgE isotype-switching. However, only direct exposure of moDCs to ovalbumin drives Th2 polarization and a humoral B cell response allowing for IgE mediated mast cell degranulation, IL13 and IL4 release in this sequential DC-T cell-B cell-mast cell model, indicating also an immunomodulatory role for IEC. This *in vitro* coculture model combines multiple key events involved in allergic sensitization from epithelial cell to mast cell, which can be applied to study the allergic mechanism and sensitizing capacity of proteins.

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**2'FL and 3FL, but not butyrate, modulate ovalbumin induced IEC-DC-T-cell activation *in vitro*, and 2'FL decreases mucosal mast cell activation in a murine model for hen's egg allergy**

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Early life is a window of opportunity to prevent food allergy. One of the most common being hen's eggs allergy, with a prevalence of 0.5-2% in infants. The immunomodulatory effect of two common fucosylated human milk oligosaccharides (HMOS), 2'-fucosyllactose (2'FL) and 3-fucosyllactose (3FL), were studied in an *in vitro* mucosal immune model using intestinal epithelial cells (IEC)/dendritic cells (DC) and DC/T-cell cocultures, and an *in vivo* model for hen's egg (ovalbumin) allergy. IEC from the *in vitro* mucosal immune model were exposed to ovalbumin, the effects of epithelial preincubation with 0.1% 2'FL or 3FL and/or 0.5 mM butyrate were studied. 3-4 Weeks-old female C3H/HeOuJ mice were fed AIN93G diets containing 0.1-0.5% 2'FL or 3FL two weeks prior to and during sensitization and challenge. Allergic symptoms, systemic and local immune parameters were assessed. Exposing IEC to butyrate *in vitro* left IEC/DC/T cell crosstalk unaffected, while 2'FL and 3FL showed differential immunomodulatory effects. In 3FL exposed IEC-DC-T cells, IFNγ and IL10 secretion was enhanced. This was also observed upon preincubation of IEC to 2'FL and butyrate, but not 2'FL alone. In presence of OVA, 2'FL enhanced downstream type 1, type 2, and regulatory responses, while 3FL downregulated type 2 and enhanced type 1 responses independent of butyrate. Butyrate did not affect OVA activation, but when combined with 3FL it provoked IL6 release from DCs ($p<0.001$). Mice fed the 0.5% 3FL diet had a lower percentage of Th2 cells in MLNs, but the humoral response was unaltered. OVA-allergic mice receiving 0,1 or 0,5% 2'FL diets had reduced OVA-IgG2a ($p<0.05$) or mast cell marker mMCP1 in serum, in association with increased caecal short chain fatty acids (SCFA) concentration ($p<0.05$). In conclusion, *in vitro* butyrate exposure promotes the development of a downstream type 1 and regulatory response in the presence of 2'FL in homeostasis but did not alter immunomodulatory effects of 3FL. 2'FL and 3FL differentially modulated ovalbumin induced mucosal inflammation mostly independent of the presence of butyrate. Dietary supplementation with 3FL decreased the Th2 frequency *in vivo* but did not affect other allergy parameters. 2'FL improved the humoral immune response and suppressed mast cell activation in association with increased SCFA production in hen's egg allergic mice.

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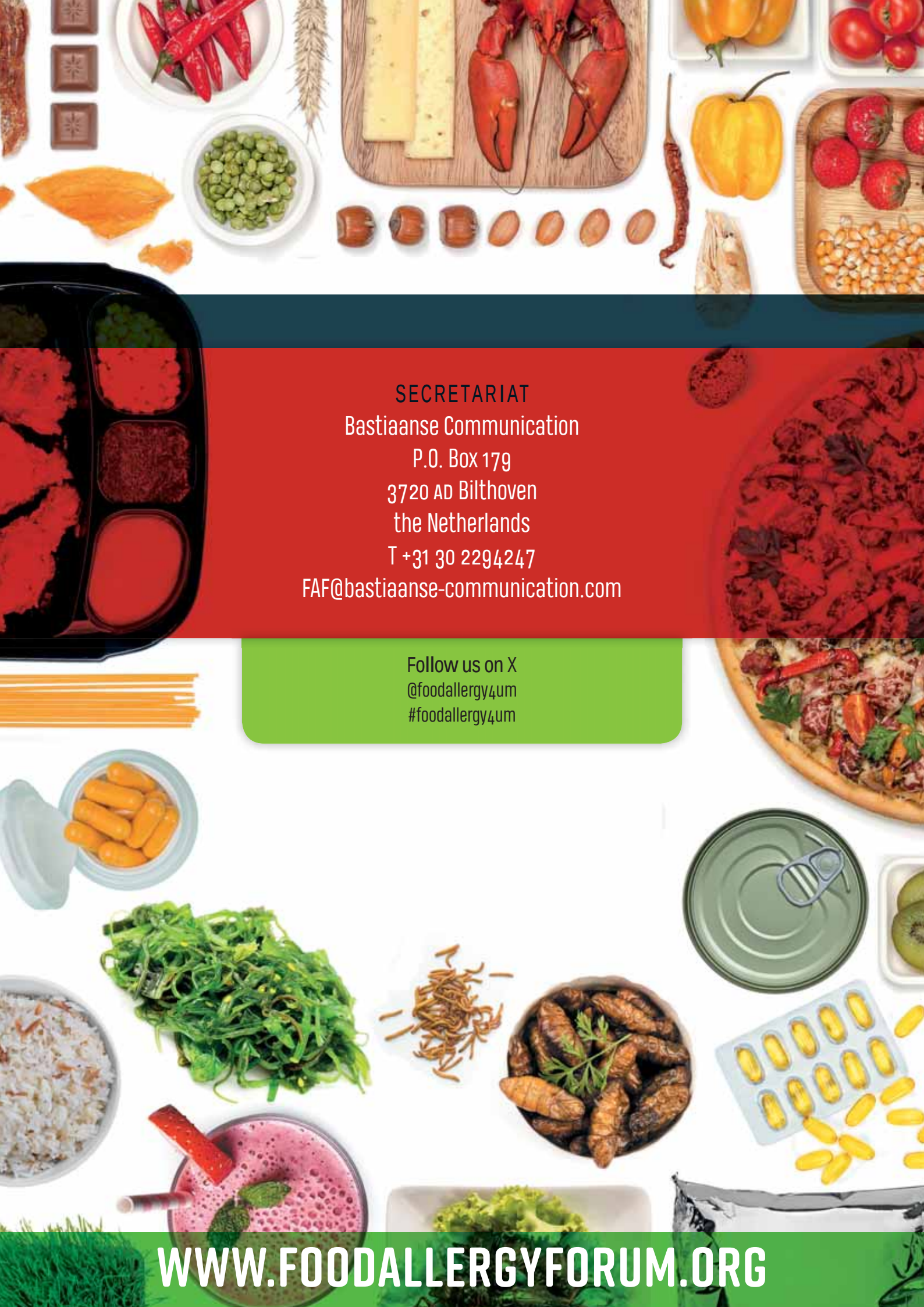


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