From EuroPrevall to iFAAM – Insights into Food Allergen Management

Clare Mills, Kirsten Beyer, Lars Poulsen, Steve Taylor, Sabine Baumgartner, Rene Crevel, Sabine Schnadt, Ronald van Ree
Food Information for Consumers Regulation 1169/2011 requires allergen information to be provided for directly sold non-packed AS WELL AS prepacked foods. It includes foods sold over the internet and through vending machines.

Substances or Products Causing Allergies or Intolerances

1. Cereals containing gluten, namely: wheat, rye, barley, oats, spelt, kamut or their hybridised strains, and products thereof, except:
   - (a) wheat based glucose syrups including dextrose (%);
   - (b) wheat based maltodextrin (%);
   - (c) glucose syrups based on barley;
   - (d) cereals used for making alcoholic distillates including ethyl alcohol of agricultural origin.

2. Crustaceans and products thereof;

3. Eggs and products thereof;

4. Fish and products thereof, except:
   - (a) fish gelatine used as carrier for vitamin or carotenoid preparations;
   - (b) fish gelatine or tigllat used as a fining agent in beer and wine;

5. Peanuts and products thereof;

6. Soya beans and products thereof, except:
   - (a) fully refined soya bean oil and fat (%);
   - (b) natural mixed tocopherols (€304), natural D-alpha tocopherol, natural D-alpha tocopherol acetate, and natural D-alpha tocopherol succinate from soya bean sources;
   - (c) vegetable oils derived phytonene and phytoether esters from soya bean sources;
   - (d) plant sterol ester produced from vegetable oil sterols from soya bean sources;

7. Milk and products thereof (including lactose), except:
   - (a) whey used for making alcoholic distillates including ethyl alcohol of agricultural origin;
   - (b) lactose;

8. Nuts, namely: almonds (Amygdalus communis L.), hazelnuts (Corylus avellana), walnuts (Juglans regia), cashews (Anacardium occidentale), pecan nuts (Carya illinoinensis (Wangenh.) K. Koch), Brazil nuts (Bertholletia excelsa), pistachio nuts (Pistacia vera), macadamia or Queensland nuts (Macadamia ternifolia), and products thereof, except for nuts used for making alcoholic distillates including ethyl alcohol of agricultural origin;

9. Celery and products thereof;

10. Mustard and products thereof;

11. Sesame seeds and products thereof;

12. Sulphur dioxide and sulphites at concentrations of more than 10 mg/kg or 10 mg/litre in terms of the total SO₂, which are to be calculated for products as prepared ready for consumption or as reconstituted according to the instructions of the manufacturer;

13. Lupin and products thereof;

Mandatory labelling of ingredients has helped allergic consumers

…….but what about all the precautionary labelling.....and “free-from” foods allergic consumers can trust?

As well as managing allergenic ingredients in recipes, what about managing the use of common utensils in fast-food outlets?
Managing food allergies needs data which have been lacking

• How many people suffer from food allergy in Europe?

• Which are the major foods?

• What is the relationship with severity/minimum eliciting doses?

• What are the risk factors/causes?

• What is the impact of quality of life?

• How much does food allergy cost society?
The Prevalence, Cost and Basis of Food Allergy Across Europe (IP, 4 years 7 months; €14.3M)

- Investigating environmental, dietary & genetic influences on food allergy.
- Delivering information (patterns & prevalence, socioeconomic cost) & new tools to improve management.

63 Partners from 23 countries including Europe (UK, NL, D, B, S, F, I, IRL, E, GR, PL, CZ, HU, IT CH, A, DK, IS, BG, RU) Africa (Ghana) and Asia (India, China) with collaborating centres from USA, Canada, NZ and Australia.
EuroPrevall Cohorts – seeking to define how many people suffer from food allergy

- Birth Cohort (Kirsten Beyer)
- Community Surveys (Peter Burney)
- Outpatient Clinic Study (Montserrat Fernandez-Rivas)
Urban and rural environments - effects on prevalence and patterns of food allergies, infections and parasitic disease
EuroPrevall – definitions applied in the cohorts

“Perceived [possible] food allergy” to any foodstuff: reported reactivity collected with the questionnaire.

“Probable food allergy” to the 24 selected foods: reported immediate reactions to a food item and specific IgE (positive SPT and/or CAP) to the same food.

“Confirmed food allergy” to the 9 foods selected for DBPCFC: reported immediate reactions together with specific IgE and a positive DBPCFC (or a positive open food challenge after a negative DBPCFC).
The cohort data shows geographic differences in incidence of allergy to cow’s milk. Cows milk allergy was highest (~1.25%) in UK, The Netherlands and Lithuania. There is very little allergy to cow’s milk in Athens. (Schoemaker et al. Allergy (2015; 70(8):963-72))
The same kind of pattern was observed for allergy to eggs.

Egg allergy incidence was highest (~2.2%) in UK.

In Athens allergy to egg affected 0.07% of infants!

How will this change in the years to come?

Wong et al Chinese University of Hong Kong, Hong Kong Unpublished data
DBPCFC: Common protocols and materials for older children and adults

Clinical leader: Barbara Ballmer-Weber; Food Materials leader: Alan Mackie

Priority 1 foods blinded in

(1) Chocolate desert (stored at room temp, just add water)
(2) Chocolate (peanut, hazelnut only)
(3) Puree desert peach and apple (freshly prepared; no threshold)
(4) Burgers and tomato sauce (shrimp)
Dosing protocol – used for ALL challenges

Dose 1: designed to give the no observed adverse effect level (NOEL) and lowest observed adverse effect level (LOAEL)

Dose 9: Equivalent to a daily serving

<table>
<thead>
<tr>
<th>Dose no</th>
<th>Mass</th>
<th>Protein</th>
<th>Matrix dose (%)</th>
<th>Cumulative Dose (mass)</th>
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<tbody>
<tr>
<td>1</td>
<td>6µg</td>
<td>3µg</td>
<td>0.0006</td>
<td>6µg</td>
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<td>2.2g</td>
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<td>5.286g</td>
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<tr>
<td>9</td>
<td>6g</td>
<td>3g</td>
<td>3</td>
<td>11.286g</td>
</tr>
</tbody>
</table>

Ballmer-Weber, Mills et al JACI 2015
Dose-response modelling using Lowest Observed Adverse Effect Levels (LOAELs) and interval censoring survival analysis

• Dose-response modelling using LOAELs from the combined population (median age 15.6) gave a ED10 value of ~3 mg peanut protein (log-normal model). This equates to ~12 mg of peanut seed.
• 95% Confidence intervals are wide (0.2, 38)
• This value is similar to the 12.3 (9.0, 16.8 95% confidence intervals) mg of peanut (median age 7) reported previously (Taylor et al Food Chem 2010)

Ballmer-Weber, Mills et al JACI 2015
Point estimates and confidence intervals for ED10s for the different foods (objective symptoms)

ED10 values for peanut, hazelnut, celeriac and fish point estimates overlap
Only shrimp is an outlier!
Integrated Approaches to Food Allergen and Allergy Management (IP, 4 years, €9M)

- Developing evidence-based approaches and tools for MANAGEMENT of ALLERGENS in FOOD
- Integrating knowledge derived from their application into FOOD ALLERGY MANAGEMENT plans and dietary advice.
- Reducing the burden of food allergies in Europe and enabling the European food industry to compete in the global market place.

36 Partners from 15 countries including Europe (UK, AU, BE, DK, CH, CR, DE, FR, IE, IS, IT, ES, GR, LT, NL, PL), Turkey and the USA and collaborators in Australia.
Objective 1: Nutrition and allergy throughout life (Module 1)
- Early life nutrition and allergy
- Dietary interventions for allergy prevention

Objective 2: Risk factors and severity (Module 2)
- Biomarkers for severe reactions to food
- Intrinsic and extrinsic risk factors

Objective 3: Validated Risk Models (Module 3)
- Modelling allergenic risk
- Clinical evidence and validation of models

Objective 4: Tools for Allergen Management (Module 4)
- Multi-analyte allergen analysis
- Clinically-relevant analysis

Objective 5: Delivery of evidence-based integrated tools for food allergen and allergy management across the food chain to key stakeholders groups (Module 5)
- Biomarkers for severe reactions to food
- Intrinsic and extrinsic risk factors
- Modelling allergenic risk
- Clinical evidence and validation of models
- Multi-analyte allergen analysis
- Clinically-relevant analysis

Allerg-e-Lab, STAKEHOLDERS (Consumers, risk assessors/managers, Industry, Health professionals)

INNOVATION, INDUSTRIAL COMPETITIVENESS AND SMEs
Early Life Nutrition and Allergy
(Kirsten Beyer with Thomas Keil and Gideon Lack)

Currently advice is that

• Women with a family history of allergy are encouraged to feed their babies only on breast milk for the first 6 months
• Introduction of allergenic foods, like milk, eggs, wheat, nuts, peanuts, seeds, fish and shellfish should be phased after 6 months of age

However, we lack the evidence on which to base such guidance!
Risk Factors and Severity
(Ronald van Ree with Montserrat Fernandez-Rivas and Lars Poulsen)

• Currently we cannot predict which individuals might experience a severe allergic reaction.
• There are three factors that appear to be involved in driving more severe reactions,
  • Food and food allergens themselves,
  • Patient-associated intrinsic factors
  • Patient-associated extrinsic factors
Bioaccessibility and extractability – the role of the food matrix in allergenicity

- iFAAM is comparing the effect of the water continuous EuroPrevall dessert with a cookie matrix n peanut challenges (Manchester, London)
- iFAAM is investigating the effect of proton pump inhibitors on walnut challenges (Madrid, Zurich)
- In FSA-funded TRACE project is investigating the effect of exercise and stress on reactivity to peanut is being studied (Clark [Cambridge], Boyle, Turner [Imperial], Mills [Manchester])
Module 3: Risk Models
(Steve Taylor with Astrid Kruizinga, Jonathan Hourihane, Rene Crevel)

Developing tools for allergen tracking and managing contamination

Linked to clinical validation through single dose challenges contamination
Single dose challenges for validation of threshold dose distributions

- Approach developed for validation of existing threshold doses for peanut (PATS)
- At the ED05 are given to potentially allergic patients
- This should give 5 reactions [with objective signs] per 100 allergic patients

Single doses of allergen are being applied in iFAAM to hazelnut, egg and milk
On-line reactions in the community

- Questionnaire developed by UCC and UNIMAN through focus groups run with Anaphylaxis Ireland and Anaphylaxis Campaign
- Tool implemented in Allerg-e-lab to collect data on allergic reactions experienced by patients
- Study running in Ireland (Allerisc) and UK (Alleric)
- A total of ~30 reactions recorded (10 in IE, 20 in UK)
Tools for Allergen Management (Sabine Baumgartner with Karine Adel-Patient)

This aspect is focussed on the
• Development of effective multianalyte analysis tools potentially suitable for in-factory testing or confirmatory in-laboratory analysis
• Defining how the performance of these tools relates to the nature of the allergenic hazard they are attempting to quantify.
Clinically relevant allergen analysis I

- IgE-mediated allergic reactions are almost all caused by the protein of foods - so methods need to be focussed on protein analysis.
- Analytical targets need to be found in problem foods as they are eaten
  - Milk – skimmed milk protein
  - Egg – pasteurised egg white (or whole egg….but how many children actually react only to yolk?)
  - Peanut – roasted peanuts more widely consumed than other forms
- Targets need to be those molecules towards which IgE-binding and activity is directed......
Clinically relevant allergen analysis II

• Working with real foods and ensuring materials are characterised regarding allergenic activity
• Evaluating the importance of matrix effects on allergenicity – helping support interpretation of test results in future [do people react to peanut in the same way in chocolate and cookies?]
• Ensuring detection limits are relevant to the levels of allergens known to cause allergic reactions …..
**Ensuring materials are clinically relevant**

Karine Adel-Patient, Hervé Bernard [INRA-CRJJ], Harry Wichers [FBR-DLO]

- iFAAM peanut flour bound well to serum IgE from peanut allergic patients;
- These data show that the ingredient contains major allergens which are IgE reactive and is suitable for use as an analytical QC material.

Similar approaches are being taken to characterise the hazelnut flour.
Current ELISA methods for allergens are able to detect low levels of allergens but may not quantify as accurately as desired.

**Egg:** one kit could determine egg protein reliably, and then only at the 3ppm level.

**Milk:**
- *Milk (casein) kits* which were less accurate and underestimated the true concentration of milk in the samples.
- *Milk “other” kits* gave results with many “extreme” values (both high and low outliers). Most kits underestimated the true concentration in the samples.

Current methodology for egg and milk will struggle to report levels of allergens in >50g servings indicated by VITAL.

Johnson et al Food Chemistry [2014] 148:30-6
Inter-laboratory trial for peanut

An interlaboratory comparison of ELISA and targeted MS analysis using triple quadrupole platform technology is being undertaken for peanut in chocolate dessert. Participants include:

- **Test kit manufacturers:** ELISA Systems., Neogen Corp., Morinaga, Romer Labs UK Ltd, and RBio-Pharm GmbH.
- **Mass Spectrometry platform vendors:** ABSciex, Waters Corp., Agilent
- **Analytical Laboratories:**
  - 20 laboratories for ELISA
  - 18 laboratories for MS analysis
  - They include laboratories in N America, Europe and Australia
- **Steering group including iFAAM partners,** Christine Parker (FDA) and Phil Johnson (FARRP-UNL)
Many challenges remain for allergen analysis

- Lack of sequenced genomes makes development of MS methods for food allergens more difficult
- Lack of reference materials and agreed ways of calculating and reporting allergen which is meaningful for everyone – including patients!
- Black box for immunoassays –
  - Unknown composition of calibrators/antigen mixture in ELISA kits
  - Antibody quality and cross-reactivity not defined hence what do labs need to check out?
- Mass spectrometry has a way to go –
  - Issues of specificity could also affect MS
  - Variability of results due to processing effects and matrix effects (and other?) effects means no single ideal extraction method for all food matrices is likely
How much is too much?
[informing what to measure and how low do we need to go]

• Helping to inform industry when to use PAL
• Helping patients to understand what PAL means for them

Measuring how much
[making sure we are measuring what is important and at a relevant level]
The Team


Campden BRI: Helen Brown

All only possible through the 63 EuroPrevall partners and collaborating centres AND those individuals who have participated in the studies!
Part-time unit using a combination of on-line teaching and a week face-to-face intensive hands-on practical training (Risk assessment, swabbing, ELISA, PCR, MS)

Course tutors include
- Professor Clare Mills
- Rene Crevel
- Professor Katie Allen
- Professor Nikolaos Papodopolous
- Dr Mike Bromley
- Dr Lee Gethings

Starting 1st July for 12 weeks with residential week 11th-1th July, Manchester, UK

Faculty of Medical and Human Sciences
For more information about the course or to register your interest, please contact:

cpdmhs@manchester.ac.uk
www.manchester.ac.uk/food-allergen-cpd

Part of a BBSRC modular training partnership with Unilever, Waters Corp and Fera Sciences