Executive Summary:
The iFAAM project set out to develop evidence-based approaches and tools for MANAGEMENT of ALLERGENS in FOOD and integrate knowledge derived from their application and new knowledge from intervention studies into FOOD ALLERGY MANAGEMENT plans and dietary advice. This was done with the primary objective reducing the burden of food allergies borne by allergic patients in Europe and beyond, whilst enabling the European food industry to compete in the global market place. This was achieved over the four year lifetime of the project, by 43 partner organisations from 19 countries, including 14 member states, Iceland, Switzerland, Turkey, The USA and Australia. Drawing on a trans-disciplinary
skill base including clinical researchers, basic scientists, psychologists, mathematicians and computer scientists. iFAAM was underpinned by the Allerg-e-lab health informatics platform for data collection and curation to allow full exploitation of complex data. The project has achieved the following:

1. **EARLY LIFE NUTRITION AND ALLERGY**: A school-age follow up of the pan-European EuroPrevall birth cohort, together with the nationally funded Irish birth cohort Baseline has been undertaken. It has shown that the vast majority of young European children with confirmed hen’s egg or cow’s milk allergy became tolerant by early school age but that other food allergies to peanut or tree nuts persisted. Coding of dietary data has been undertaken and analysis shown differences across Europe with regards breastfeeding and infant feeding patterns. The pooled analysis of data from four trials investigating whether early introduction of peanut and hen’s egg has confirmed reductions in peanut allergy but current evidence on early egg introduction and egg allergy prevention is less clear and recent trials have reported contrasting results.

2. **RISK FACTORS AND SEVERITY**: Mining the historic EuroPrevall data sets has allowed a numerical score for severity of reactions to be developed which has been validated in other cohorts together with candidate biomarkers and risk factors of severity. A study of intrinsic and extrinsic factors that may modify severity suggest that the food matrix and viral infections may modify sensitivity, whilst ant-acids, such as proton pump inhibitors have no significant effect on threshold dose.

3. **RISK MODELS**: A suite of tools have been developed for food allergen management and using data on threshold doses form allergic individuals and consumption data from the general population. Tools include an allergen tracking tool, the iFAAM Tier 1 and Tier 2 risk assessment tools, together with a risk mitigation matrix. However, there is only sufficient data to provide the most reliable reference doses for six foods – peanut, hazelnut, celery, shrimp, egg and milk whilst for others, notably many tree nuts, data are completely lacking. Approaches for clinical validation have been shown to be effective for hazelnut and cow’s milk. Working with patient organisations a study of allergic reactions in the community have shown a small number of individuals are responsible for the majority of incidents and application of iFAAM tiered risk assessment approach it showed that in all cases where a food sample was analysed that the exposure was potentially unsafe with Tier 2 indicating a minimum risk of unexpected reaction of 20% in allergic user population. A shortened version, AllerREACT, was rolled-out to patient groups and indicated that pre-packed food was responsible for reactions in 24% of cases for both adults and children with the majority of reactions taking place in the home for children but only around half for adults.

4. **ANALYSIS OF ALLERGENS IN FOOD**: Mass spectrometry methods developed using the iFAAM peptide collection, provided an effective prototype multi-analyte screening method for detection of the five allergenic foods in two different “hard to analyse” chocolate containing matrices. Quantification is more challenging but certain peptide targets in particular matrices show promise to allow quantification of allergens at the same sensitivity as immunoassay methods. An international ring trial was subsequently undertaken with more than 20 participants from Europe, Australia, Japan and North America. Results for the ELISA arm indicate that all immunoassay kits underestimated the level of peanut protein incurred with a wide variation in recoveries. The MS method allowed detection of peanut at a similar level to the ELISA. Investigations into how food matrices affected the extractability, bioaccessibility and bioavailability of allergens gave contrasting results with the chocolate bar being the least bioaccessible.

5. **FOOD ALLERGEN AND ALLERGY MANAGEMENT KNOWLEDGE BASE**: Stakeholders interaction was integrated into iFAAM to deliver harmonised integrated approaches, including risk assessors and managers managing population risk, the food industry who manage allergens to ensure consumer safety, health care practitioners to provide food allergy management plans and dietary advice and allergic
consumers to manage individual risk. This was achieved through workshops, dissemination of tools through the internet and publications.

Project Context and Objectives:
The last 30 years has seen a rise in the incidence of allergic disease, including IgE-mediated food allergy, such that it is now estimated that around 5-7% of infants and 1-2% of adults suffer from this condition. A variety of environmental and dietary factors may play a role in determining susceptibility to allergic disease, with events early in life being thought to be especially important. In many countries, national recommendations have been made on how to feed infants to reduce the risk of food allergy; these recommendations vary widely reflecting the lack of firm evidence.

Currently there is no accepted cure for food allergy. Consequently, individuals with food allergy often have to practice life-long stringent avoidance of foods to which they are sensitised, and their quality of life is impaired. Those at risk of severe reactions must carry rescue medication in case of accidental consumption of their “problem” food. However, managing food allergens to avoid their unintended presence in products where they are not part of the recipe remains an issue for the food industry. In order to develop more meaningful food labelling an evidence base is urgently needed to identify what the generally safe level of allergen contamination is and derive action levels. These will allow allergens to be managed more effectively by targeting resources and thus minimising costs.

The overall objective of the iFAAM project is to develop evidence-based approaches and tools for management of allergens in food and integrating knowledge derived from their application into food allergy management plans and dietary advice delivered by health care practitioners thus providing holistic strategies to reduce the burden of food allergies in Europe and beyond whilst enabling the European food industry to compete in the global market place. We will develop the evidence-base and scientific opinion required to develop recommendations on introduction of allergenic foods into the infant diet in order to prevent development of food allergies later in life.

The iFAAM overall objective has been addressed by developing evidence-based tools and approaches which will enable the various stakeholders to manage food allergies in an integrative way through an integrated set of specific objectives through a set of interacting subsidiary objectives as follows:

Objective 1: Assess the influence of maternal diet and infant feeding practices (including weaning) on the patterns and prevalence of allergies across Europe to develop the evidence base to support development of strategies for allergy prevention (Module 1)

Obj 1.1 Undertake standardized follow-up assessment at school age in the EuroPrevall multicentre birth cohort, which is critically important to capture clinically meaningful outcomes in older children, not only in food allergy, but also other atopic diseases (asthma, rhinitis and eczema). When combined this will present an integrated data set on allergy outcomes running from birth to the age of ~10 years and will provide data on the prevalence of clinical reactions to individual food allergens.

Obj 1.2 Within the EuroPrevall multicenter birth cohort a tremendous amount of data on maternal diet and infant feeding practices has been gathered, together with data on early development of other atopic diseases such as eczema, rhinoconjunctivitis and asthma. iFAAM will capitalize on this by applying the multilingual indexing software tools (LanguaL, developed in the EuroFIR project) to achieve a consistently indexed dataset of food consumption across the EuroPrevall cohort.

Obj 1.3 Bring together the data from several dietary intervention studies aimed at food allergy prevention to provide new scientific knowledge and inform the development of EU dietary guidelines. Specifically, data from randomised controlled trials on prevention of peanut allergy through early introduction of peanut
in the UK (Learning Early About Peanut Allergy, LEAP) and Germany (Preventing Peanut Allergy in Atopic Dermatitis, PEAAD), similar studies utilising egg in Australia and Germany (Hens Egg Allergy Prevention, HEAP) along with a randomized controlled trial of early introduction of allergenic foods to induce tolerance in infants (Enquiring About Tolerance, EAT) in the UK, will be brought together.

Objective 2: Establish risk factors determining the development of severe reactions to food in food sensitised individuals, and identify associated biomarkers (Module 2)

Obj 2.1 Build on the EuroPrevall cohort of severely reacting individuals, supplementing it with additional patients from iFAAM clinical studies. This cohort of well-defined patients will be used retrospectively in an integrated fashion to identify intrinsic risk factors including patterns of sensitisation (to foods and allergen components) and linking them with biological factors determining elicitation of allergic reactions at the cellular level (mast cell biology). We will also seek to extend a previously established European-wide anaphylaxis registry through the iFAAM partnership.

Obj 2.2 Investigate the role of extrinsic factors affecting severity of reactions through food challenge and other studies. This will include an investigation of how the food matrix, together with co-factors such as non-steroidal anti-inflammatory drugs and antacids, that may modify the ability of allergen molecules to elicit allergic reactions by modifying their release from foods and uptake into the circulation. Complimentary investigations on the uptake of allergens by epithelial surfaces in the oral cavity will be undertaken to assess along with their biological activity which may govern potency of allergic reactions, including effects on gene expression. Lastly the modifying effect of viral infections on reactions to foods will be assessed by skin testing, a direct measure of tissue-associated mast cells. This will build on synergies with the EU-funded project PreDicta which focuses on the role of viral infections in allergic and associated respiratory disease.

Objective 3: Develop a clinically validated tiered risk assessment and evidence-based risk management approach for food allergens and evidence based risk management of the presence of unintended allergens in the food chain (Module 3)

Obj 3.1 Using data (published and unpublished, from national and European studies such as EuroPrevall) reference values for allergenic ingredients listed in Annex IIIa (Directives 2003/89/EC and 2007/68/EC amending Directive 2000/13/E, Annex II of Food Information Regulation) will be derived based on dose distributions of individual thresholds. These will be linked to representative consumption models in order to build robust, probabilistic risk assessment algorithms for management of allergens in Europe which will be trialled using allergen contamination scenarios for the most important production processes. Assessments will be made to determine for which allergenic foods on Annex IIIa existing data are sufficient to establish reference values and to identify those foods, like tree nuts other than hazelnut, for which data gaps exist. Since walnut was identified as an important allergenic food in the EuroPrevall project a multi-centre study will be undertaken to provide missing data on dose distributions for this food.

Obj 3.2 Dose-distribution models characterising population response to allergens will be clinically-validated using a single dose challenge protocol with respect to major, widely used allergenic ingredients, including cows’ milk, egg, peanut, hazelnut and celery. Probabilistic risk assessment models will be validated through a pilot study seeking to estimate the overall frequency of all reactions experienced in a free-living group of allergic patients. This study will be developed in collaboration with allergic patient groups and informed by complementary on-going nationally-funded studies.

Objective 4: Allergens in Food: Clinically relevant multianalyte analysis (Module 4)

Obj 4.1 We will provide food manufacturers with an integrated effective tool box of multi-analyte immuno- and mass spectrometry methods for detection and quantitation of allergens in foods. This will focus on
development of common extraction protocols and use of clinically-validated matrices as check samples and establish proof-of-concept for (1) peanut-treenuts (at least hazelnut-walnut) and (2) egg-milk, as exemplar multianalyte targets that present the food industry with allergen management problems and for which well validated multianalyte tools are lacking. Immunobased tools will be developed suitable for both on-site testing explicitly linked to mass spectrometry methods for in-laboratory confirmation of test results for raw materials through to finished food products.

Obj 4.2 For analytical tools to effectively support food allergen and allergy management it is essential they are fit for purpose in terms of sensitivity, specificity and precision and that they provide meaningful data that can be related to the inherent allergenicity of an ingredient in a given food matrix. This is more complex for allergens than for other small molecule chemical hazards because the nature of the hazard (i.e. the allergen) is not as well defined. For example, allergens may adopt different forms following food processing (either different conformations or chemically transformed by reaction with, for example, sugars, to form Maillard adducts). The processing-induced changes and the matrix formulation may modulate the extractability and bioavailability of allergens from foods as well as their inherent allergenic properties. We will address this by relating detection of allergenic ingredients by mass spectrometry and immuno-based tools to their allergenic activity, alone and after formulation into the model matrices, capitalising on the resources generated by iFAAM clinical studies (Objectives 2, 3). One element will address whether the apparent reduced extractability of allergens from the cookie reference material developed by the MoniQA project is reflected in a reduced capacity to elicit an allergic reaction in peanut allergic individuals. This will enable us to, uniquely, ensure the biological validity of the methods developed for allergen detection and provide clinical verification of a relevant reference material.

Objective 5: Delivery of evidence-based integrated tools for food allergen and allergy management across the food chain to the key stakeholder groups (Module 5)

Obj 5.1 Allergen analysis tools will be integrated with data and risk models to develop a comprehensive allergen management toolbox that will enable management of allergens across the food chain in a more cost-effective and sustainable manner. Emphasis will be placed on the usability of tools for SMEs and associated training in their application across Europe and to major global centres that represent regions where European industry both imports raw materials and exports finished foods. Tools will be made available on websites such as those established through previous projects (MoniQA, EuroPrevall). The application of such approaches will allow manufacturers to manage allergens more effectively minimising the use of precautionary labelling and developing clear guidelines for its use, making it more transparent than at present.

Obj 5.2 Build a holistic approach to food allergy management spanning dietary strategies for prevention of allergies, through to managing food avoidance, and identifying those at risk of severe reactions and equipping them to manage their condition more effectively. This will include the role of early introduction of allergenic foods, and assess the role of food processing on the allergenicity of infant foods and how food allergen management plans may need to be adapted for the special needs of infants. Such data will be assembled in to an evidence base which can readily contribute to the development of dietary advice to pregnant mothers and guidelines on weaning and infant nutrition regarding allergies.

The effective management of food allergens and food allergies requires active involvement of all stakeholders, all of whom need to be informed by accurate information on either allergen content in food and the risk it poses of triggering a reaction, or given dietary advice to prevent their development in the first place. These include

• Regulatory authorities who manage the risk at population level including national authorities, European
risk managers e.g. DG SANTÉ and risk assessors e.g. the European Food Safety Authority (EFSA) and equivalent authorities around the world such as the US Food and Drug Administration and HealthCanada.

- Food industry to manage allergens in food to ensure consumer protection
- Health care practitioners to provide food allergy management plans and dietary advice
- Allergic consumers to manage individual risk

iFAAM has built on existing EU networks and investment, notably the EuroPrevall and MoniQA projects, and integrate the tools developed through the EuroFIR project for coding dietary information. The outputs of iFAAM and the integration it will achieve will enable the EU to maintain its world leadership in the field of food allergy, deliver improved consumer protection and support industrial innovation. We will build on e-Health concepts to allow us to exploit fully the complexity of population-based data, maximise sharing and linkage of data, and to develop data storage through the development of an informatics platform “Allerg-e-lab”. This will underpin the iFAAM research strategy by supporting the collection of data and providing a platform for researchers to access the data and share their research activities and discoveries through the following:

- An online archive for storage of and access to completed EuroPrevall studies
- A web-based set of questionnaires for in-clinic collection of new follow-up data
- A web-based tool for collection of patient reported outcomes data for the community study
- A networked resource for sharing consistent information about the data and data extracts, algorithms and models developed between researchers.

The iFAAM overall objective will be addressed through an integrated set of specific objectives through a set of interacting subsidiary objectives delivered through five complimentary Modules.

Project Results:

MAIN S&T RESULTS/FOREGROUNDS

EARLY LIFE NUTRITION AND ALLERGY (MODULE 1)

The effect of early life nutrition on food allergy has been investigated in two ways. The first has involved undertaking a follow-up of the pan-European non-interventional EuroPrevall birth cohort (1, 2). Coordinated by the Charité in Berlin it was originally formed of nine centres from Germany, Iceland, Lithuania, Poland, The United Kingdom, The Netherlands, Spain, Italy and Greece and common questionnaires were implemented in a sister cohort in Ireland, Baseline, with national funding. The cohort has shown there are marked geographic differences in the prevalence of food allergy in infancy and early childhood, which is much lower in southern European countries, such as Greece, compared to north-western European countries, such as The Netherlands and the UK (3, 4). Data were collected in the cohorts on maternal diet during pregnancy and early life, giving the opportunity, through a school age follow-up assessment, to investigate how early life nutrition might influence the temporal development of food allergy.

Eight of the centres, together with the Irish Baseline cohort, were able, through the iFAAM project, to fully participate in the school-age follow up. The centres worked together to develop the harmonized standardized questionnaires, along with a manual for the clinical follow-up. This included the development of the Allerg-e-lab in-clinic data collection tool in the form of an online questionnaire targeting the whole population sample and forms for collecting data on investigations done during the clinical visit. Much effort was devoted to the development of an improved food challenge protocol, which was being used as a common form across all challenges undertaken in iFAAM (5).

As with many epidemiological studies, response rates varied across different countries (Figure 1) with the lowest (44% response rate) in the UK and the highest being in Iceland with a response rate of more than
The high response rate in Cork, Ireland, was likely because the children were younger (3-5 years old) and not yet in school and hence it was easier to convince families to participate. Three centres (Berlin, Germany; Amsterdam, The Netherlands; Reykjavik, Iceland) were able to access additional support. Lower than average response rates were accounted for by centres with mobile populations, migrating away from the study region, often due to economic crises and new job possibilities elsewhere (e.g. in Athens, Greece; Madrid, Spain; and Vilnius, Lithuania). In the UK centre, families had been recruited from a large urban-rural area, which made it more difficult for families with school-age children to participate. The average response rate across the entire EuroPrevall cohort was 58.3% and including the Irish Baseline cohort, was 61.1%. This is consistent with school-age follow-ups of other European population-based birth cohorts.

Blood samples were taken as part of the clinical follow-up of the cohort, which established a serum bank of 1,750 samples at AMC (Amsterdam, Netherlands). This was used to undertake a detailed serological analysis across the cohort to generate information on allergic sensitisation to:

**Food allergens:**

1. Peanut and tree nuts (pecan, pistachio, walnut and cashew) together with peach to total extracts as these are important food allergens in older children.
2. Major allergens associated with a greater risk of more severe reactions - Ara h 2 for peanut, Cor a 14 for hazelnut, Pru p 3 for peach and Ana o 3 for cashew.

**Inhalant/aero-allergens:**

1. Birch pollen, grass pollen and house dust mite which are important causes of allergic rhinoconjunctivitis and allergic asthma and are a potential source of cross-reactivity to foods.

All the iFAAM follow-up study data are captured within the Allerg-e-lab platform and data cleaning has been completed. In addition all the historic EuroPrevall study data sets have been uploaded into Allerg-e-lab to facilitate data curation, harmonisation and analysis.

These data sets have been used to describe the natural history of food allergy from the age of 3 years up to school-age with the aim of investigating how tolerance to some allergenic foods (such as cow’s milk and hen’s egg) develops in some, yet may persist in other individuals as well as emerging food allergies associated with adulthood. As expected, the vast majority of young European children with confirmed hen’s egg or cow’s milk allergy became tolerant by early school age. Other food allergies such as against peanut or tree nuts seem to persist more often, i.e. in about 50% of the cases, until school-age and possibly beyond. Further follow-up in adolescence and adulthood, including confirmation of food allergy by double-blind food challenge tests as the current diagnostic gold standard, is needed to characterise the course of childhood food allergy in the long-term.

In parallel with planning and undertaking the school age follow-up, and to enable effective interrogation of dietary data in the EuroPrevall and Baseline cohorts, approaches were explored to recode the data on maternal diet and early infant feeding. The data were consolidated into a standardised dataset to characterise food consumption during (a) pregnancy and (b) the first 12 months of life. The same approach has also been applied by EuroFIR on the iFAAM follow-up using the dietary data collected from a standardized food frequency questionnaire. LanguaL coding was used, and the food composition information was extracted from EuroFIR FoodExplorer, an online accessible database which allows the simultaneous search of 28 standardized and specialized food composition databases (FCDB) from most of the EU countries, as well as Canada and USA. LanguaL is based on the concept that any food can be systematically described by a combination of characteristics and that the characteristics can be
categorized into viewpoints and coded for computer processing for retrieval. In general, the more processed a food becomes; the more details are required to adequately describe it. In addition, a composite food inherently requires more details than a single food. From this point of view, the LanguaL system was designed specifically for description of foods and is therefore best suited where comprehensive and flexible description of foods is needed.

The nine European birth cohort centres differed in their feeding practices regarding breastfeeding and the introduction of complementary foods to the infant’s diet. Although the majority of mothers initiated breastfeeding in all nine centres, the duration of any breastfeeding and the prevalence of full breastfeeding differed to a great extent between the study centres. For instance, a high prevalence of any breastfeeding for at least 4 months was found in the Icelandic study centre (>90%), whereas prevalence was rather low in the Greek (<51%) and Dutch (<57%) study centres. The high prevalence estimate of breastfeeding in Iceland has been reported before in the literature.

Evaluation of the school-age follow-up assessment of the birth cohort will improve our understanding of the natural course of early life food allergy. It will help paediatricians and nutritionists to give parents of young children with food allergy better information on the prognosis of their child’s disease, i.e. the likelihood that the child will “grow out” of the allergy by school-age (as is often the case for cow’s milk and hen’s egg) and the importance of regular high quality diagnostic tests of food allergy with a higher potential to persist to school-age and possibly beyond. Furthermore, early life predictors, risk and protective factors for food allergy in school-age can now be evaluated for a number of social, lifestyle, environmental and biological parameters. In particular, factors that are potentially modifiable can then be studied further in specifically controlled randomized intervention studies to develop evidence-based preventive strategies for future generations.

A second strand of investigating how diet can influence development of food allergies later on in life has been the linking together of data from seven international randomised controlled trials (RCTs) on timing of introduction of allergenic foods for

- Peanut allergy: LEAP (UK), PEAAD (Germany),
- Hen’s egg allergy: HEAP (Germany), STAR (Australia), STEP (Australia), BEAT (Australia)
- Various common food allergens including peanut and hen’s egg: EAT (UK)

Approaches to data harmonisation were developed to enable the data to be uploaded into the Allerg-e-lab platform, which will support meta- and pooled analyses. This activity required a variable mapping exercise to be undertaken, to identify aspects of the trials that could be compared and identify specific outcome measures and meta-data which could be combined (Figure 2). The pooled analysis of data from four RCTs looking at early introduction of peanut and hen’s egg provides data from a large number of individual children and increases the power, to analyse sub-groups with different eczema severities, ethnicities and ages at introduction in comparison to the individual trials. The high number of diagnoses based on food challenge also gives robust estimates of allergy prevalence and the harmonisation of criteria used to diagnose food allergy and sensitisation adds to the robust data presented. These analyses showed reductions in peanut allergy on an intention-to-treat basis and for hen’s egg allergy on a per-protocol basis between groups randomised to “early” introduction of peanut and hen’s egg respectively in comparison with those asked to avoid these foods. The results of this pooled analysis provide new evidence for the efficacy of early introduction of peanut in children with all degrees of eczema including those without eczema and lend support to the recent recommendations on the early introduction of peanut in all children by the US National Institute of Allergy and Infectious Diseases. However, the current evidence on early egg introduction and egg allergy prevention is less clear and recent trials have reported contrasting results.
In addition, serum samples from approximately 1300 children enrolled in the EAT study at 3 time points were analysed at AMC. IgE results from the EAT study were combined with those from the LEAP, BEAT and HEAP studies to investigate whether the early introduction of food reduces sensitisation to egg at 12 months of age and peanut at 3-5 years of age. Analysis of the EAT data undertaken so far indicates that it mirrors the findings of the LEAP study, confirming the well-known discrepancy between sensitisation and clinical reactivity to foods, a reduction in IgE production in the intervention group only becoming apparent after 2.5 years of age. It will be important to follow this cohort to determine whether the intervention influences IgE production as the children become older.

Individual trials across Europe and internationally have reported conflicting results on the influence of early introduction of allergenic foods on food allergy prevalence. The pooling of trial data is an essential component to fully understanding the role of early introduction of allergenic foods in reducing the prevalence of food allergies in children. The results from this pooled analysis will add substantially to the evidence upon which new and revised guidelines for allergy prevention can be based.

RISK FACTORS AND SEVERITY (MODULE 2)

The starting point of Objective 2 was to reach consensus about a method to score severity of IgE-mediated adverse reactions to food through a workshop held with the iFAAM clinical partners in collaboration with the UK Food Standards Agency-funded TRACE project partners. This contributed to developing a consensus on the methods to be used and the items to be included in the development of a severity score. Thus the following factors were used:

1. Clinical manifestations of the reactions according to the target organ/system involved: oropharyngeal area, skin, eye (conjunctiva, C), nose (Rhinitis, R), larynx, lung (bronchospasm, BR), gastrointestinal (GI), cardiovascular (CV) and neurological (N) systems;
2. Amount of the trigger food needed to induce a reaction;
3. Onset of reaction;
4. Treatment received.

Two approaches were developed to scoring severity by SERMAS. The first qualitative (ordinal) score involves classifying the severity of reaction into five grades according to the target organ(s) and system(s) involved and ranges from mild reactions restricted to the oropharyngeal mucosa with the most severe reactions involving the cardiovascular and ventral nervous systems. A second, numerical scoring system was also developed using mathematical modelling. The qualitative grading system and the numerical score had to be equivalent, the numerical score having been designed such that the sums of values of symptoms in a certain qualitative grade would not be higher than the lowest value of a higher (more severe) grade. In summary, the Food Allergy Severity Score (FASS) developed in iFAAM has two formats, an ordinal one (iFAAM-oFASS) and a numerical one (iFAAM-nFASS). Both the oFASS and nFASS have followed internal validation in the EuroPrevall outpatient clinical data set (2121 patients and 8258 different food reactions). For the internal validation the need for emergency room assistance (or hospitalization) and medication (alone or in combination: adrenaline, corticosteroids, antihistamines, IV fluids, oxygen, vasopressors) were used as indicators of severity. By means of logistic regression and bootstrapping it was shown that the oFASS and nFASS were able to predict severity.

The ordinal score (iFAAM-oFASS) was then applied in the EuroPrevall outpatient clinic (6) data sets which were mined to identify biomarkers and risk factors associated with severe reactions by AMC. The data set categorized by the iFAAM-oFASS was subsequently mined to identify biomarkers and risk factors associated with severe reactions by AMC. This has utilised data, such as patients’ demographic and clinical characteristics, and biomarkers, such as specific serum IgE (sIgE) against whole extracts from 24...
allergenic foods, 12 inhalant allergen sources and latex and against 53 individual allergen molecules (known as component resolved diagnosis – CRD) from a selection of these foods and inhalants. A number of risk factors and biomarkers were identified, using different bio-statistical methods, classical univariate and multivariate approaches as well as data mining approaches. This has shown that predicting severity is a complex matter, and that generalization for all foods is not possible (7). A more detailed analysis was subsequently performed on the hazelnut data set of EuroPrevall that included 731 patients (8, 9). Symptoms (reported during double blind placebo controlled food challenge, DBPCFC) were categorized as being either mild (grade 1), moderate (grades 2 and 3) or severe (grades 4 and 5). Multiple regression models to predict severity were generated from clinical factors and sensitization patterns (extract and allergen/CRD based). Reactivity to Cor a 9 and 14 was positively (OR 10.5 and 10.1 respectively), and Cor a 1 negatively (OR 0.14) associated with severe symptoms during DBPCFC, with areas under the curves (AUCs) of 0.70-0.73. A combination of Cor a 1 and 9 improved AUC to 0.76. A model using a combination of atopic dermatitis (risk), pollen allergy (protection), IgE against Cor a 14 (risk) and walnut (risk), increased the AUC to 0.91. At 92% sensitivity, the specificity was 76.3% and the positive and negative predictive values 62.2% and 95.7%, respectively. For reported symptoms, associations and generated models proved to be almost identical but weaker. In summary, the model combining CRD with clinical background and extract-based serology was found to be superior to CRD alone in assessing the risk of severe reactions to hazelnut, particular identifying those at low risk of having a severe reaction. The iFAAM oFASS and nFASS have subsequently undergone external validation by SERMAS using a collection of more than 3,500 reactions collated from a variety of data sets including the European Network of Severe Allergic Reactions (NORA, (10)), the EuroPrevall community survey (11), the EU SAFE project data on apple allergy (12), projects on egg (OVALE) and milk (CoALE) funded by the ISCIII of the Spanish Ministry of Science, together with the iFAAM data sets. After undergoing external validation the FASS was shown to predict severity in different populations. The role of non-IgE isotypes (IgG, IgG4 and IgA) in severity of allergic reactions to peanut has also been investigated by AMC using a set of serum samples from the OUH biobank. These serum samples originated from 137 challenged peanut-sensitized subjects with either proven tolerance (n=25) or varying severity grades of peanut allergy. IgE against peanut, and in particular against its major allergen Ara h 2, has previously been shown to be a biomarker for peanut allergy and for its severity. Despite that, not all patients with significant IgE titres against Ara h 2 had severe symptoms. The hypothesis was that non-IgE isotypes may explain this variation, acting as protective, so-called blocking, antibodies. Such a protective role has been established for IgG4 responses generated during allergen-specific immunotherapy and more recently during early life intervention studies such as the LEAP study (WP2), seeking to prevent development of allergies. Despite this evidence there is an on-going debate as to whether non-IgE isotypes are actively involved in protection against allergies or whether they are merely a read-out of allergen exposure. Our investigation demonstrated that non-IgE isotypes are in fact closely associated with IgE antibody responses (consistent with their being subject to similar immune regulation processes). However, they did not contribute to predicting either tolerance or severity in peanut allergic patients, neither as an isolated biomarker nor as combined biomarker expressed as ratios with IgE. The mathematical modelling undertaken on large data sets has been complemented by a series of mechanistic studies investigating the effects of intrinsic biological characteristics that may predispose towards severe reactions, and a selection of extrinsic factors, such as the use of medications like proton pump inhibitors, the food matrix and viral infections. Biomarkers that are bedded in mechanisms of disease are more powerful than markers identified simply by association. While iFAAM and many previous
projects have investigated the use of specific IgE as such a marker, it is clear that also other factors play a role. Thus, the effector cells (mast cells and basophils) involved in orchestrating allergic reactions were studied in detail in iFAAM. The investigations of mast cells and basophils have taken two directions. Firstly, using biological samples from a small cohort of well-characterized patients, a back-to-back comparison of histamine release techniques with basophil activation tests was undertaken and showed there was no difference in cellular characteristics between food allergic patients and non-allergic subjects. Secondly the same patient material was used to investigate, for the first time, whether haematological stem cells differentiated in vitro would be give rise to a different mast cell phenotype in food allergic compared to non-allergic control subjects. This turned out not to be the case, which suggest that either such differences do not exist or comparisons should be made either using new in vivo methods or by ex vivo studies where mast cells are freshly isolated from buccal tissue. The last mechanistic study developed and validated an non-invasive procedure for collection of material from the oral mucosa that was applied to patients undergoing DBPCFCs. This biological material was further analysed by a full transcriptomic analysis, and while a few differentially expressed genes were found, the overall impression is that the epithelial cells of the oral mucosa are not sufficiently activated – at least sufficient to mount a gene expression and protein production – to detect by this method.

The widespread use of proton pump inhibitors (PPI) has been proposed to play a role in the increase of food allergy and to possibly affect severity of reactions by lowering thresholds. The rationale behind this hypothesis is the effect of PPIs on the pH in the stomach, i.e. causing an increase to values where pepsin becomes less efficient in digesting proteins including allergens. Using walnut as a model allergenic food, a clinical study has been undertaken by UZH and SERMAS to establish the impact of PPI use on the threshold dose for walnut. The results suggest that there is no significant effect on threshold dose, but that there may be effects on severity of reaction. To establish the impact of different food matrices on threshold dose and severity of reaction, a comparison of reactivity to peanut has been undertaken, using a group of patients previously challenged at Imperial with peanut in the EuroPrevall dessert matrix through the UK nationally-funded TRACE project. These patients have been re-challenged using the iFAAM cookie matrix containing peanut. This has provided a comparison of allergenic activity of roasted peanut in a water continuous matrix, which comprises a starch gel matrix with only 6% (w/w) oil, with a 20% (w/w) fat baked matrix. Dose distributions will be compared with other challenges undertaken with the iFAAM cookie and implementation of the iFAAM severity scoring system will allow the impact of both the PPIs and the food matrix on clinical reactivity to foods, to be established. For the peanut challenges the patterns of reactivity in terms of threshold dose and symptom severity will also be assessed against uptake of allergen into the circulation.

In (allergic) asthma it is well established that common respiratory tract virus infections may trigger exacerbations. Here we were interested to establish whether this is a more common phenomenon that may also have impact of food reactions. In the project it was not possible to evaluate that by doing food challenges in children with and without common viral respiratory infections. As a proxy to a clinical response during a challenge we set out to assess the impact of such infections on skin reactivity to (food) allergens, using skin prick tests (SPT). Allergic children were enrolled before the winter peak of common viral infections to undergo evaluation by SPT. Parents were then asked to come back when the child had a common viral infection. Although it turned out difficult to reach the power originally planned for this study, UoA managed to evaluate sufficient children to demonstrate that skin reactivity increases during the viral infections. Future studies will be required to establish whether this increased sensitivity also translates into
decreased thresholds and/or increased severity of reactions to food.

RISK MODELS (MODULE 3)

Allergens are an unusual food hazard in that they are harmless to more than 95% of consumers and are often used in considerable quantities in food manufacturing, factors that make them a challenge to control in manufacturing operations. In practice it is not possible to exclude allergens completely from foods formulated without a particular allergenic ingredient when shared facilities and processing lines are used. This has required the development of approaches to quantify the risk posed by unintended allergen presence, as a basis for allergen management strategies to assure safety. Unlike chemical food hazards, where safety data are obtained in animal models and then extrapolated to humans, the lack of adequate animal models for food allergy has necessitated the use of data obtained from low dose oral food challenges undertaken for diagnostic or clinical research purposes in food allergic individuals. The individual no observed and lowest observed adverse effect levels obtained in patients have been used to derive dose distributions of minimum eliciting doses in populations allergic to a number of common allergenic foods and allow eliciting doses (EDs) to be calculated for different proportions of the population.

Through the activities of TNO and FARRP a large data base of oral food challenge data on different foods from published clinical studies was assembled before the start of the iFAAM project (13). These data have been enriched with data from the EuroPrevall studies (8), and the collective datasets further reviewed and refined to give a full dataset comprising data from 2000 individuals (Table 1) for allergens on the EU list of priority allergenic foods (cashew, celery, egg, fish, hazelnut, lupin, milk, mustard, peanut, sesame, shrimp, soy and wheat).

From this evidence base it is clear that data gaps exist, notably there is a complete lack of threshold data for many tree nut species included on Annex 2 of the EU Food Information for Consumers regulation (i.e. pecan, pistachio, cashew, Brazil nut, Macadamia nut), different species of fish, crustacean and molluscan shellfish. Only six foods – peanut, hazelnut, celery, shrimp, egg and milk, have more than 60 subjects included, the ideal number required for dose distribution modelling to provide the most reliable reference doses (14). Other data gaps include the effect of subject age, the problem that for some allergens data may originate largely from a single clinical centre (e.g. cashew data). Other data have a restricted geographic origin although this may also reflect the prevalence of an allergy (e.g. subjects reacting to celeriac (celery spice) largely originate from Switzerland). Selection bias in the patient populations undergoing challenges is also a concern with the possibility of individuals with a history of severe reaction being excluded from challenge due to safety concerns. However, the majority of studies and hospitals included in this analysis did not exclude individuals with a history of severe reactors. Studies such as immunotherapy clinical trials, may also introduce bias through skewed selection of patients reacting at certain doses as part of their inclusion criteria. These concerns can best be addressed by continuing to collect data from worldwide clinical centres and comparing dose distributions of groups based on different selection criteria.

Since walnut is an important allergenic tree nut for which data were lacking a dose distribution study was undertaken in iFAAM which also delivered data on the impact of a proton pump inhibitor intervention. The dose at which 10% of patients responded with objective allergic symptoms to walnut (also known as the ED10) was 4.7 - 9.4 mg walnut protein, depending on the statistical distribution. The data are comparable to values determined within the EuroPrevall project and Taylor et al. (2014), with objective symptoms recorded ED10 values for hazelnut and peanut (8, 13) whilst another recently published study on walnut described ED10 values of 10-14.6 mg protein (15).

An important aspect of developing reference doses that might act as benchmarks for allergen
management, including precautionary allergen labelling is to undertake clinical validation. An approach was developed for validation of the peanut dose estimated to cause a reaction in 5% of the allergic population (ED05) based on the data used by the Voluntary Incidental Trace Allergen Labelling (VITAL™) Scientific Expert Panel, using a single dose challenge (16). This study design was adopted and used to undertake single-dose trials for hazelnut, cow’s milk and egg with ED05 values of 1.5 1.0 and 0.5 mg protein respectively. It had also been the intention to undertake such studies for celery (celeriac or celery tuber a major component of celery spice) and walnut but data to derive meaningful ED05 values for single dose challenges were not available. The single dose challenges undertaken for hazelnut and milk have provided important pilot data and indicate that further validation using additional subjects will enable the ED05 values to be fully validated. However, none of the 75 children recruited into the study reacted to the ED05 for egg. This could be interpreted to suggest the estimated and tested ED05 is lower than the actual ED05. There are a number of possible explanations for this difference, including selection of a too conservative an ED05 value due to a different age of population studied or variation in the forms of egg used in the studies from which the egg ED05 was estimated compared to the pasteurised egg white powder used in iFAAM. Raw egg was used as the challenge material with many of the subjects included in the original VITAL data set (14). However, raw egg eliciting doses would also not provide insights into the issues of managing allergenic food ingredients in food manufacturing since pasteurised egg ingredients are always used.

The development of risk models also requires data on consumption patterns. National food consumption data from DK, FR and NL coded with FoodEx2 were made available in kind by DTU (DK), ANSES (FR) and RIVM (NL). FoodEx2 was chosen as the food coding system since this made the datasets from different countries comparable. All the food types that may be contaminated with an allergenic food ingredient during processing were included in the survey. Since food allergic reactions happen rapidly following an eating occasion this was taken as the relevant consumption parameter to consider. Data were grouped according to food item and consumption on both a country-by-country basis and as a combined data set, which allowed 56 food groups to be identified. Whilst data from the French Nationally funded MIRABEL project provided important insights into food consumption patterns of peanut allergic individuals, the small number of control subjects in the study meant it was not possible to draw conclusions on the food consumption of peanut allergic patients compared to the general population. Consequently the national food consumption data from Denmark, France and The Netherlands were used to create a common set of food consumption data (consumption in a meal) and group them to be used in the development of the risk assessment approaches.

The data on threshold doses and consumption were then used as the basis for developing the iFAAM Tier 1 and Tier 2 risk assessment (Figure 3). Also an allergen tracking tool and a risk mitigation matrix were developed.

Allergen tracking tool: An important aspect of managing allergens in foods is to understand how allergens may enter food ingredients across the supply chain and then how food manufacturing processes may result in allergen contamination. To that end an allergen tracking tool was developed which can estimate the probability of unintended allergen presence through identifying the unintended presence of allergen and then undertaking a vulnerability assessment based on the production steps. An extension of the tool covers a vulnerability analysis of the supply chain.

Tier 1 risk assessment: The Tier 1 risk assessment has been developed to ensure the output is appropriately conservative with the option to include an uncertainty factor for the contamination parameter. The concentration of unintentional allergen (in mg total protein of the allergenic food) present in a food
product is an important component in the risk assessment. It is obtained by determining allergen levels either in product samples or samples taken at intermediate steps in the manufacturing process. As an alternative to analysis, a simple method has been developed for estimating the level of unintended allergen presence using information such as the allergen content in the preceding recipe and estimating the carry over into the next production batch. During the course of iFAAM, and based on data coming from the iFAAM allergen analysis Module 4, it became apparent that the analytical data contributed the greatest uncertainty and variation to outcomes. This results from factors such as sample preparation, extraction methods, analytical kits used and their performance. Consequently the risk assessment has been designed to have flexibility with regards the uncertainty factor used for allergen analysis. With regards inclusion of consumption data a sensitivity analyses was performed to determine the most optimal point estimate for food consumption taking into account that the Tier 1 results should be safe for the majority of the allergic consumers. If the Tier 1 tool shows that the potential allergen exposure in a given product is above the reference dose, mitigation measures have to be employed and the cycle repeated. If the problem persists, Tier 2 risk assessment should be applied and if mitigation proves insufficient, a precautionary allergen label applied.

Tier 2 risk assessment: Probabilistic risk assessment approaches for food allergy have been developed based on either second order Monte Carlo simulations (TNO; (17)), or a combination of Bayesian inference and second order Monte-Carlo simulations (ANSES; (18)). An alternative approach combining frequentist inferences and second order Monte-Carlo has also been developed to investigate if a non-Bayesian approach is able to also take into account uncertainty. These models use the same input variables of food consumption (i.e. how much of the suspected contaminated product is consumed), allergen concentration (i.e. how much allergen is in the contaminated product) and threshold distribution (i.e. how much allergen do the allergic consumers react to). However, they use the data in different ways as the TNO model does not actually integrate the risk uncertainty whilst the ANSES model makes explicit the uncertainty propagation from the input variables to the risk calculation. A comparison of the methods has been undertaken using a peanut allergic population consuming cereal bars employing consumption data from Denmark, The Netherlands and France. Four case studies developed based on previous publications were used to estimate and compare the risk of unexpected reactions. Contamination models for the Tier 2 risk assessment have been based on case studies being performed to examine unintended allergen presence in the production process. The estimated risk of reaction per eating occasion was found to be similar with the different simulation approaches, ranging from 9.8% to 14.7%, with the differences being attributable to the use of different distribution models (lognormal versus Weibull curve fitting procedures). The details of different probabilistic models used by TNO, FARRP, and ANSES were found to provide consistent risk estimate and therefore formed a consensus approach.

Risk mitigation matrix: The risk mitigation matrix summarizes risk management measures taken from the literature, guidelines and practice. Measures are organized by location in the process or facility. An indication of cost and efficacy of the measure has been added to allow the best choice based on needs and resources.

An important aspect of food allergen management is having an understanding of the context within which food allergic individuals experience allergic reactions. To that end a questionnaire was developed and evaluated for assessing allergic reactions within free-living communities of allergic patients in the UK (AlleRiC) and Ireland (AlleRiSC) in collaboration with the iFAAM Patient stakeholder group (19). In the UK 29 reactions were reported by 15 of the 72 subjects recruited; approximately one third of reported
reactions were to curries/Asian cuisine (all catered), one third to chocolates or desserts and the remainder to a mixture of other food types. In the Irish study there were eleven incidents reported by six of the 58 participants with an even split of reactions between manufactured and catered/home prepared foods. Where possible food samples were obtained and the content of suspected allergen determined. The data were then analysed using the iFAAM tiered risk assessment. Most of the reactions recorded were experienced through the presence of high levels of allergen, often from catered foods, rather than pre-packed, manufactured foods. When used to test the iFAAM tiered risk assessment approach it showed that in all cases the Tier 1 risk assessment indicated that the exposure was potentially unsafe and either a refined risk assessment (Tier 2) or risk mitigation was needed. Tier 2 indicated a minimum risk of unexpected reaction of 20% in allergic user population. This also leads to the conclusion that risk mitigation is needed.

The AllerRiC and AllerRiSC tool was subsequently used by UCC to develop a shortened on-line reporting tool, AllerREACT, in collaboration with patient groups from Spain, France, Poland and Germany (DAAB) (20). This tool collected data on 677 incidents from 628 subjects and indicated that pre-packed food was responsible for reactions in 24% of cases for both adults and children with the majority of reactions taking place in the home for children but only around half for adults.

ANALYSIS OF ALLERGENS IN FOOD (MODULE 4)

Determination of allergens in foods is a critical aspect of effective food allergen management, since it underpins the risk assessment process through effective monitoring of cleaning and mitigation steps to minimise unintended allergen presence, as well as monitoring and enforcement by regulatory agencies. There has been a need for an alternative complementary method for allergen analysis to immunoassay for some time, which targeted mass spectrometry (MS) analysis has great potential to meet. Thus, a major goal of Module 4 was to develop an effective integrated tool box of multi-analyte immuno- and mass spectrometry methods for detection and quantitation of allergens in food (Figure 4), assuming that the detected allergens are still clinically relevant, i.e. able to elicit an allergic reaction. Allergen detection in different matrices was then related to their in vitro bioaccessibility and in vivo bioavailability.

In order to ensure that analysis was focussed on relevant targets, major allergenic food ingredients were sourced including skimmed milk powder, pasteurised egg white powder, mechanically-defatted lightly roasted peanut flour, and hazelnut flour, all of which were used for oral food challenges in iFAAM. In addition a source of food-grade walnut flour was also identified. All the ingredients were first characterised biochemically with regards to their protein contents. They were then incurred into three different complex matrices – the EuroPrevall chocolate dessert matrix, the iFAAM cookie matrix (also used for food challenges) and a chocolate bar.

A crucial first step was to optimise allergen extraction which was undertaken in a highly systematic manner exploring different buffer systems (such as ammonium bicarbonate and Tris-borate buffers), pH values, times and temperatures of extraction and buffer:food ratios. It became apparent that the conditions required for effective protein extraction and subsequent analysis using MS workflows was not compatible with immunoassays. Therefore the conditions were optimised for the different methods in parallel. A second aspect of the MS workflow was the need for digestion with a specific endoprotease, trypsin and therefore optimisation of digestion conditions was undertaken with regards to trypsin type, protease-to-protein ratio, temperature and presence of detergents. Based on the results from these studies optimised conditions for extraction and digestion of the five different allergenic ingredients in the three different matrices were finalised.
Using the optimised sample extraction and preparation protocols peptide targets were identified with a view to developing a multianalyte method for the quantification of the five food allergens. It was decided the most appropriate platform for this was a triple quadrupole instrument platform, using multiple reaction monitoring (MRM) experiments. A total of 101 candidate peptide targets were identified by BOKU, PEI, JRC and UNIMAN using a combination of different types of mass spectrometry platform technology (21). Thorough analysis of data for all y- and b-ion fragments led to the identification of up to four possible transitions for each of the 101 peptides. From this peptide set 30 suitable candidate peptides were selected and synthesised as unlabelled or isotopically labelled peptides for final assay development and as quantification tools. Following optimisation of the chromatographic and MS parameters serial isotopic dilution series (SIDs) were set up to determine the limit of detection and limit of quantification of each peptide in blank (0 mg/Kg) allergenic ingredient. Subsequently chocolate bar and chocolate dessert matrix, as representatives of complex food matrices, incurred with 0, 3, 10, 30 and 100 mg protein/kg for each of the five allergenic ingredients and analysed using the MS methods showed that peptide reporters could detect allergenic ingredients in the range of

• Chocolate bar – detect all allergens from 1-13 mg/Kg and quantify 2-13 mg/Kg protein
• Chocolate dessert - detect all allergens from 2-19 mg/Kg and quantify from 6-54mg/Kg protein

Thus the MS methods developed using the iFAAM peptide collection, provide an effective prototype multi-analyte screening method for detection of the five allergenic foods in two different “hard to analyse” chocolate containing matrices. Quantification is more challenging but certain peptide targets in particular matrices show promise to allow quantification of allergens at the same sensitivity as immunoassay methods.

The immunobased method was developed in parallel as validation of the food allergen multiplex immunoassay was done by INDOOR. The assay utilizes Luminex xMAP® technology that is based on the use of allergen specific antibodies that allows for simultaneous quantification of up to 100 proteins. It was optimized to ensure sensitive and reproducible detection of allergens from peanut, egg, milk and shrimp together with hazelnut. Quantification is allowed thanks to purified allergens as standards. Focussed on specific allergenic molecules these immunoassay methods provide a direct comparator to the mass spectrometry methods.

An international ring trial was subsequently undertaken to compare the performance in term of reproducibility and sensitivity of peanut allergen analysis by immunoassay and mass spectrometry for peanut. Building on the MFAN industrial stakeholder group, it has involved more than 20 participants from Europe, Australia, Japan and North America, including vendors of ELISA test kits and mass spectrometers, as well as analytical service laboratories and academic researchers. Incurred materials were used spanning allergens at levels between 4-40 mg peanut protein/Kg food identified within Module 3 as being important for allergen management. For the ELISA arm a total of 18 laboratories and five of the ELISA test kit manufacturers took part in the full ring trial. The EuroPrevall chocolate dessert matrix was used to incur roasted peanut flour at levels of 0, 2, 4, 10 and 30 mg peanut protein per Kg of reconstituted dessert matrix and analysed using five different commercially available immunoassay kits. The lowest limit of detection for these immunoassay kits ranged from ≤ 0.025mg/kg peanut protein with the highest at 0.625 mg/kg peanut protein, using the conversion of 25% protein in whole peanut kernel for the kits reporting in whole peanut. Results for the ELISA arm indicate that all immunoassay kits underestimated the level of peanut protein incurred into dessert matrix after reconstitution and with a wide variation in recoveries. Lower levels of recovery were observed compared to a previous ring trial, specific to immunoassays testing for egg and milk incurred into the same chocolate dessert matrix. The poor
recoveries by the ELISA test kits may in part be explained by the difficulties in extracting proteins from processed foods using buffers compatible with ELISAs.

For the MS arm of the ring trial, a total of 12 laboratories and two MS vendors completed in the MS arm of the peanut allergen analysis preliminary ring trial. Participants used a variety of chromatography set-ups and MS platforms, highlighting differences in MS instrument sensitivity in the detection of peptides. Out of the five peptides monitored in the pre-ring trial, three showed promise as targets for the detection of allergenic proteins in food. All participants detected the light and heavy peptides at similar ratios across the entire SID concentration range. Participants using particular MS platforms were able to identify 2 peptides in the peanut-incurred chocolate dessert matrix with some achieving quantification even at the 4 mg/Kg peanut protein level. Certain combinations of micro-flow LC separation and MS platform show the potential for MS methods to quantify peanut protein at the 4mg/Kg peanut protein level in a chocolate containing matrix. The protocols were refined ahead of and similar data were obtained in the full ring trial.

To ensure that allergens detected are clinically relevant and then correspond to a real allergic risk, peanut and hazelnut ingredients were also characterised with regards their allergenic activity, both alone and, for peanut, after inclusion in the different matrices. This was undertaken by determining the IgE-reactivity of food using serum samples from peanut and hazelnut allergic subjects. Complementary methods were combined: IgE immunoblotting, IgE-immunoassays (IgE binding capacity) and in vitro models of elicitation of the allergic reaction, using various cell lines derived from mastocytes or basophils. After ensuring the allergenicity of the ingredients, the effect of incurring the peanut flour in the three matrices (chocolate bar, EuroPrevall chocolate dessert or iFAAM cookie) on the extractability of IgE-reactive proteins was assessed. This showed that the IgE binding to peanut proteins was retained in the food matrices but that the matrix composition affects both extractability and allergenic activity of peanut allergens. The chocolate bar had the most significant effect on the IgE-reactivity and extractability, followed by the EuroPrevall chocolate dessert matrix and then the iFAAM cookie. In parallel, cell-based assay for assessment of IgE-functionality of variously processed food products in various matrices, was optimized and shown similar effects.

Subsequently, the relationship between peanut allergen extractability and detection from the different matrices was compared to peanut allergen bioaccessibility and bioavailability. Bioaccessibility is a property which reflects the impact the food matrix has on the release of a molecule (in this instance, allergens) from the food matrix in the lumen of the gastrointestinal tract. This can be modelled using in vitro digestion systems. In contrast bioavailability is a property related to the uptake of a molecule by the gastrointestinal mucosa and its subsequent appearance in the circulation (Figure 5)

An assessment on the impact of the matrices on bioaccessibility was undertaken using an in vitro model of digestion including a model chew, simulated gastric and intestinal digestion under “normal” conditions and when intragastric pH is raised to 6.0 following use of antacids (Figure 6). Using this digestion model the resistance of peanut proteins, including peanut allergens, to digestion was compared when in the form of the roasted peanut flour ingredient and after inclusion into the EuroPrevall chocolate dessert matrix, and iFAAM cookie. Resistance to digestion was monitored using a combination of SDS-PAGE, immunoblotting with allergen-specific antibodies and IgE reactivity using IgE immunoblotting and histamine release assays. These studies showed that there was little impact of the matrices on allergen accessibility and that the 2S albumin allergens, Ara h 2 and 6, were highly resistant to digestion.

One factor that could affect allergen solubility, and possibly bioaccessibility and bioavailability, is the interaction of allergens with plant polyphenols found in many foods, including cocoa. Since cocoa phenolics are not widely available, model polyphenols were obtained from a commercial source (rutin,
quercetin and epigallocatechin gallate). An analysis of molecular interaction between polyphenols and purified peanut allergens was undertaken together with the impact of their inclusion in a chemically-modified starch gel matrix. The molecular interaction of the polyphenol with the purified allergens was weak and had little effect on the extractability of peanut proteins, their detection and quantification by several different peanut ELISA test kits and their IgE reactivity or their bioaccessibility and digestion. The in vitro studies on bioaccessibility were complemented by an investigation in healthy volunteers on the effect of matrices on the uptake of allergens into the circulation, i.e. their bioavailability. An immunoassay, capable of detection of very low levels of the major peanut allergen Ara h 6 (a 2S albumin allergen highly resistant to digestion) was validated for analysis in serum. During the course of the validation it became apparent the circulating immunoglobulins in human serum samples were interfering with the detection of peanut allergens. Consequently a novel serum sample preparation protocol was developed and optimized to overcome this interference. The assay was subsequently applied to the analysis of serum samples from healthy volunteers after they had consumed peanut flour alone and after it had been included in either the iFAAM cookie or the EuroPrevall chocolate dessert matrix. These data demonstrated that there is a difference in bioavailability of allergens depending on the matrix, with peanut Ara h 6 being less bioavailable after inclusion in the gel dessert, then in the cookie and finally in the roasted peanut despite being equally bioaccessible in the in vitro digestion model. This suggests that there are more complex factors mediating differences in uptake beyond availability of allergens in the gut lumen.

Threshold data have been obtained in an oral food challenge study for peanut incurred in the iFAAM cookie matrix in almost 40 peanut allergic subjects in the UK (UNIMAN, Imperial) and Switzerland (UZH). Data analysis involves dose distribution modelling and an assessment of severity of reaction using the iFAAM oFASS to compare data obtained for peanut using the EuroPrevall chocolate dessert matrix. Threshold dose and symptoms severity will be related to allergen bioavailability and allergenicity.

FOOD ALLERGEN AND ALLERGY MANAGEMENT KNOWLEDGE BASE (MODULE 5)

Delivery of evidence-based integrated tools for food allergen and allergy management across the food chain to the key stakeholder groups was a major objective of iFAAM. The first aspect was to translate tools and approaches developed in Modules 3 and 4 into food allergen management strategies which could be applied in the food industry, and particularly to support the SME sector.

The first activity to be undertaken was to undertake an analysis of food allergen recalls based on publicly available information on food allergen recalls in Europe, North America, Hong Kong, Australia and New Zealand (22). This was done to help focus activities in WP5 and WP9 on the real problems being faced by the food industry. Comprising over 2000 entries covering a 4-year period from 2011 to 2014 the database showed that the major cause of product recalls and/or alerts was undeclared allergens in either prepared dishes and snacks (12–53% of recalls/alerts), or cereals and bakery products (14–25% of recalls/alerts) (Figure 7) (22). The most important food allergen was milk and milk products which accounted for 16–31% of all products with recall or alert, followed by cereals containing gluten (9–19%), soy (5–45%), and egg and egg products (5–17%). The vast majority of the recalls (between 42–90%) were as a result of labelling errors. However, 0–17% of products with recalls/alerts could be coded as caused by the unintended presence of an allergen as the probable result of cross-contact in production. Construction of the database of allergen recalls has provided some important lessons and recommendations to the authorities in terms of the harmonisation of the reporting of allergen recalls into a more standardised format (22).

Subsequent activities were focussed around engaging with the different stakeholder groups and discussing the development of the iFAAM tiered risk assessment approach. A series of workshops was
organised by ILSI. The first entitled “Expert group and workshop on tiered risk assessment approaches” was held in Brussels on 2nd March 2015. With 59 participants from eleven member states, Switzerland, Canada, The USA and Australia the aim of the workshop was to identify whether the tiered risk assessment approaches being developed in iFAAM addressed the needs of the relevant stakeholder groups, including the food industry, patient groups and the regulatory authorities. The workshop provided important input from the stakeholder groups which supported finalization of the iFAAM tiered risk assessment approach.

This was subsequently followed by a stakeholder workshop entitled ‘Application of Food Allergen Management Tools’ took place on 25th -26th April 2016, at the EC JRC-IRMM (Geel, Belgium). In total, 46 participants attended this workshop, with representation from 11 different member states, the USA. The stakeholder groups included health care practitioners and patient organisations, public health risk assessors and regulators, with representatives from EC JRC-IRMM, EFSA, the UK FSA together with other national food authorities; and food industry and analytical sciences experts. The overall objective of this workshop was to seek critical feedback on the iFAAM risk analysis tools and approaches proposed for managing the risks posed by the presence of unintended allergens in foods. This included

- Presentation of the tiered risk management tools developed during iFAAM, showing how they can be integrated in managing the risk from unintended allergen presence;
- Demonstrating how testing methodology can be embedded within the risk assessment and management process, including best practice with regards application of allergen testing;
- Explaining how the integrated risk assessment approach can help to define benchmarks for PAL and make them transparent for all stakeholders.

An important aspect of these activities was the development of cost assessment tool in the form of a spreadsheet, which was tested as part of the case studies. The aim was to identify risk management measures and costs, assuming high-, medium- and low cost scenarios. This was done to evaluate whether the approach matched EU SMEs’ needs was value to them and pinpoint characteristics of participating food businesses that impact costs (high/ medium/ low). It also helped to confirm what constituted best practice in risk management of allergens, and defined an approach for future use of the cost-effectiveness tool. The conclusions from the activity with the case studies were that:

1. Most of the SMEs did not implement the high cost-high effectiveness approaches (i.e. dedicated sites, dedicates lines), meaning there is unlikely to be any momentum or need for them. On the other hand, most food businesses are likely to meet or exceed food safety requirements through high-medium cost-medium effectiveness measures, often already in place. In some cases, further improvements are possible via fine-tuning medium cost-medium effectiveness options, although high cost options might ultimately be required. For SMEs not meeting food safety requirements in this regard a cultural shift is required and, some medium cost options may not be feasible or high cost decisions may still be required for legal compliance.
2. The iFAAM tier 1 risk assessment approach was fit for purpose for SME use although, as expected, some measures were not relevant and the level of implementation of existing measures needed prior assessment.
3. Food manufacturers with effective wet cleaning have little scope for further improvement, apart from fine-tuning procedures, or high cost options, such as separate lines, for which there is no obvious incentive. Similarly, the procedure for biscuits had little scope for improvement, although analytical findings – which are recommended alongside Quality Managers’ estimates – above limits of detection may provide further incentive. Thus, many SME food businesses are expected to have borne most costs and only moderate costs are likely to be imposed through adoption of risk-based allergen management. In contrast,
dry mix manufacturers, given challenges in cleaning, may have exhausted medium cost options, preventing them from removing precautionary allergen labelling (PAL), based on no detectable levels (although risk assessment at Tier 1 may prove satisfactory). If they may conclude that PAL cannot be removed via risk assessment or high costs. Finally, poor allergen practices – likely with many micro- or mini-SMEs in Europe – requires improvement but medium cost measures may be precluded by core practices, and some high costs (e.g. no rework or complex management of rework, overhaul of practices) can be expected, which could constitute an important barrier to removing PAL.

4. The cost-effectiveness tool should be used in context with independent and expert knowledge of a manufacturing site to identify and review measures in place and identify others that are relevant and feasible. For example, detailed cleaning protocols might be in place but could be improved, resulting in better outcome than more expensive solutions that are not already in place. The proposed approach is, therefore, to verify whether food safety requirements are met, via the allergen tracking tool and iFAAM Tier 1 risk assessment before considering alternatives and then select measures, which may have high cost implications, to meet food safety requirements more consistently. When food safety requirements are met, other measures can be identified, considering financial constraints and goals (eliminating PAL, no detectable allergens, free from claims), starting with low-, medium- and then high cost-high effectiveness options.

Early in the project lifetime a consensus paper was developed bringing all the iFAAM stakeholder groups to consider different facets of PAL (23). Being able to make the right and safe food choice is a major issue in the daily management of their allergy for patients with food allergies and their carers. Uncertainty regarding unintended allergen presence prevents them from making informed choices. Not only patients themselves but also health care professionals, regulators, food industry and auditors face different aspects of the same problem. It was evident from this activity that, in its current form, PAL is counterproductive for food allergic consumers. Summarising the perspectives of all the key stakeholders (including clinicians, patients, food industry and regulators), with the aim of defining common health protection and risk minimization goals, it was evident that the lack of agreed reference doses has resulted in inconsistent application of PAL by the food industry and in levels of contamination that prompt withdrawal action by enforcement officers. There is a poor relationship between the application of PAL and the presence of allergen and, consequently, the actual risk of allergic consumers experiencing an allergic reaction. This reduces consumer trust in food labels and their ability to make informed choices.

In order to understand more about industry attitudes of food businesses (manufacturers, retail and ingredient suppliers) to reference doses, risk assessment and phrases to be used in precautionary allergen labels a survey was undertaken. A questionnaire was compiled determining broadly company activities and allergen management practices such as allergen testing of incoming ingredients or finished products, application of PAL, barriers and enablers to change in application, particularly the use of reference doses, and preference for alternative phrases and symbols for PAL. Made available in English, French, Italian and Polish, more than 300 responses were received from across the world, including the USA, Canada, Australia, South Africa, Switzerland, Uruguay, French Guiana, Nigeria, India, Malaysia, Jordan, and 12 EU Member States. Although invited to take part, responses were notably absent from Eastern European countries and new Member States. Participants included manufacturers, suppliers, importers, retailers and food services. In general, the food businesses that responded had a good understanding of reference doses and trusted the science, but were concerned about liability, low perception of consumer understanding, and perceived analytical uncertainty. They were also confused regarding regulation, particulate contamination, risk assessment and cost. Similarly, conflicting responses
were obtained from the food businesses that responded about the benefits of strategies, such as VITAL™. Although they were not concerned about either the costs or time required to complete the assessment and did not think it complicated, they did not perceive any additional benefit, need or applicability of VITAL™. The fact the scheme is not endorsed by regulatory authorities was a barrier for its uptake. Possible phrases and text that could replace/ be used with “may contain” were overwhelmingly rejected and it was clear from many of the answers that the industry perceives that “may contain” was an effective phrase for PAL.

A survey about PAL was also undertaken amongst consumers who are food allergic or parents/ guardian/ carers for children who are food allergic. The aim was to understand more about their attitudes to: (1) precautionary labelling, particularly how it is used, (2) potential use of reference dose for precautionary labelling decisions and (3) possible phrases and text that could replace/ be used with “may contain”, and facilitate some degree of comparison between these responses and those from the food businesses. The primary aim of the survey was to understand more about the attitudes of food allergic consumers and parents/ guardians/ carers of food allergic children to:

- Precautionary labelling, particularly how it is used
- Potential use of reference doses for precautionary labelling decisions
- Possible phrases and text that could replace/ be used with “may contain”

There were a total of 1582 responses from English-speaking respondents, mostly from Ireland and the UK, German-, Dutch-, Polish- and Spanish-speaking nationals.

Key conclusions from the survey were that a significant number of food allergic consumers perceive that no PAL meant products were safe to eat, those who never consult food labels being the most confident about consuming foods with no PAL. There was a view that if the application of PAL was underpinned by a risk assessment it would increase trust, particularly for parents and those with ‘low confidence’ in the safety of manufactured food products.

Both the surveys were presented at the final iFAAM stakeholder consultation held in Winchester (UK) on 13th-14th December 2016. Entitled “Making “may contain” transparent”. The main objectives of the workshop were to:

- Review and finalise a short guide for patients and healthcare professionals about current precautionary allergen labelling;
- Develop a proposal to improve the approach to precautionary allergen labelling for consumers.

A total of 37 participants attended with representation from ten member states and the USA. Representatives included healthcare practitioners, patient organisations, regulators and both the food industry and analytical services industry with representatives from EC JRC-IRMM, the UK Food Standards Agency and the Irish Food Safety Authority.

Other activities of the Knowledge Base have focussed on dietary risk factors for the development of childhood food allergy. This activity has focussed on bringing researchers together from across the project to discuss the evidence base for preventing food allergy through dietary means and has resulted in a consensus publication (24). Recommendations and guidelines on the prevention of food allergy have changed in recent decades especially with regards to dietary manipulation in food allergy prevention. This can encompass preventive strategies involving allergen avoidance, early introduction of allergenic foods into the infant’s diet, general consideration of infant nutrition and supplements including pre- and probiotics. The partners concluded that whilst allergen avoidance strategies should not be used for allergy prevention, a lack of consensus remains with regards what other actions should be recommended beyond exclusive breastfeeding for the first 4–6 months of life. Following a workshop on prevention held on 12th
December 2016 in London, hosted by the UK Food standards Agency, a further update based on the outputs from Module 1 activities is planned as they are published.

The second topic focused on management strategies to help protect food allergic individuals at risk of severe reactions. A state of the art review (25) has been published building on Module 2 activities, including the workshop to develop a consensus with regards severity scoring. The publication discusses the perceptions of severe, life-threatening allergic reactions to food by the different iFAAM stakeholder groups and review the evidence regarding the different factors that are thought to be involved in driving severe reactions and could potentially be used to identify those at most risk of experiencing them. For example, whilst many food-allergic children also have asthma, this is not, on its own, predictive of severe reactions such as anaphylaxis. Furthermore, it emphasises the relationship between allergen exposure and symptom severity is unclear and it appears that food allergic patients with a history of having severe reactions such as anaphylaxis, do not react at lower doses than those who do not. The group concluded that it was important to consider severity of reaction and sensitivity (i.e. the amount of an allergenic food required to cause a reaction) as separate factors.

ALLERG-E-LAB HEALTH INFORMATICS PLATFORM

In order to realize the potential of previously collected population-based data in EuroPrevall, and RCTs investigating the early introduction of allergenic foods such as EAT and LEAP, together with new data collected in iFAAM a health informatics platform, Allerg-e-lab, has been built, the first of its kind devoted to food allergy (Figure 8).

Development of the platform has entailed

- Creation of data collection interfaces with 19 e-clinical record forms able to collect more than 3,000 data items with more than 30,000 completed records included in Allerg-e-lab from the iFAAM studies. This has been complemented by the AlleRIC online interface for the allergic reactions in the community project which enabled patients to record information about allergic reactions they experienced in their everyday life (26).
- The development of data descriptions and import of data into the Allerg-e-lab Variable Bank. In collaboration with partners the metadata required to import these have been defined. This has entailed the creation of Variable Report Models that capture the relationships between variables and provide context to ensure the correct interpretation of the data. It also involved defining terms that can be related to the data to formally specify the data semantics.
- Data descriptions have been created that reference the Variable Report Models and terminology and that are used by the eLab to create Variable Reports when the data are imported. These Variable Reports can be searched, browsed and exported through the Allerg-e-Lab Cohort Manager. The Variable Report Models, terminology and data descriptions form an important milestone. They will be the starting point for an on-going process to refine the way that food allergy data are shared between computer systems and are made available to analysts in future.

Development of an overarching governance structure through the Allerg-e-lab agreement which involves owners of the data from iFAAM and EuroPrevall with an oversight management committee which will oversee the access to and use of the data in future beyond the lifetime of iFAAM, realising the project partnerships ambition to realise the potential of e-Health approaches to big data science through the effective curation of the valuable data sets developed in these projects. This will support re-use and further analysis in the years to come.

References


Potential Impact:

The potential impact of the iFAAM project

Contribution to Food, Agriculture and Fisheries, and Biotechnology Priority thematic Areas of FP7

Delivering the new knowledge and tools developed by iFAAM into the relevant stakeholder communities has been a key goal of the project. A major contribution to this has been realized through the workshops convened by ILSI. These have cut-across and involved all stakeholder groups, as well as reaching beyond the project partnership. One example is the expert group and workshop on tiered risk assessment approaches in food allergy which took place with 57 participants, a significant representation from European Member States (a total of 13) as well as the USA, Canada and Australia. Stakeholder groups included food authorities, with representatives of EFSA, FDA, UK FSA, Health Canada, ANSES, DGAL; academia; NGOs (e.g. patient organisations); and food companies. The feedback from these events has been vital in obtaining feedback on proposed approaches and tools. In addition, the discussions and outcomes were shared beyond iFAAM through trade associations, such as the UK Food and Drink Federation. Further dissemination beyond iFAAM is also envisaged with a proposed ILSI-Europe led second symposium on “Frontiers in Food Allergen Management” to disseminate all aspects of
iFAAM activities to the relevant scientific communities, including the food industry.

IMPACT ON PUBLIC HEALTH: The World Health Organisation and other food safety authorities recognise food allergy as a significant public health concern due to its prevalence, the potential severity of the condition and the impact it has on the quality of life of allergic consumers. In the UK it is the causes 65% of hospital admissions due to food ([https://www.food.gov.uk/sites/default/files/fifth-csa-report-allergy.pdf](https://www.food.gov.uk/sites/default/files/fifth-csa-report-allergy.pdf)). iFAAM has produced data and tools that will help support public health authorities in addressing the issues posed by food allergies. This impact has been realised throughout the lifetime of the project through the Regulatory authorities stake-holder group, led by DTU (involved in risk assessment of foods in Denmark) together with ANSES and the UK Food Standards Agency. The group has participated in iFAAM workshops, and has included representatives from EFSA, other member states (Sweden, Ireland, France and Germany), Health Canada and the US FDA.

iFAAM also provided input into the public consultation on the EFSA opinion (EFSA supporting publication 2014:EN-696). iFAAM outputs directly address the priorities for a socially inclusive and healthy Europe through the following aspects:

- **Updating allergen labeling lists:** The list of allergens that must be labeled at whatever level of their inclusion included in Annex II of the Food Information for Consumers Regulation, was originally drawn up based on a consensus of what were considered prevalent allergens, many of which are “hidden” in foods and could cause severe reactions. However, data to document inclusion were limited. Through the follow-up of the EuroPrevall and Baseline cohorts new data have been generated which broadens the evidence base regarding the prevalence of food allergies across Europe at different stages of the life course. Such data will help support EFSA to revise and update the list allergenic ingredients in Annex II.

- **Threshold doses:** Whilst gaps remain in our knowledge of threshold doses for foods such as certain tree nut species a large body of data is now available for peanut, hazelnut, egg and milk, with the largest ever database of 2,000 threshold doses having been produced by FARRP and TNO. Furthermore, an approach to the clinical validation of reference doses derived from such data which are likely to protect the vast majority of consumers, has been demonstrated within a Pan-European context which can enable support the development of evidence-based action levels for allergens in foods. This will help regulatory authorities (risk assessors and risk managers) to consider what might constitute appropriate reference doses for these foods.

- **Reactions in the community and acceptable risk:** The on-line reactions in the community study and associated on-line tool provide a means for monitoring reactions in the community. It provided evidence in the UK that there are significant failures in patient allergen management and allergen management in the catered food sectors. Such data provides direction for targeting scarce resources more effectively (e.g. in better training patients, improved training of food business operators especially in the hospitality and fast foods sectors). This tool could be adapted in future to provide much-needed surveillance of allergic reactions in the community, to monitor the impact of interventions such as changes in allergen labeling and introduction of new foods.

- **Severity of reactions and acceptable risk:** Predicting those at risk of severe reactions is an important aspect of public health. The iFAAM ordinal Food Allergy Severity Score (oFASS) provides a novel numerical approach to grading severity which will have many potential applications in public health from assessing how interventions (preventive, or treatments) may reduce severity of reactions, as well as providing a more objective means of assessing severity when considering which foods should be included in priority lists for allergen labeling. The tools and knowledge gained on factors affecting severity of reactions, such as the food matrix, can also be included into risk models. Furthermore the single dose
challenges provide a means for regulatory authorities, working with the patient groups, to identify whether a candidate reference dose can provide sufficient protection for the majority of allergic consumers and hence an acceptable level of risk of reaction. These single low doses may also be used clinically to identify the most sensitive food-allergic subjects (those reacting to the lowest doses) in an efficient, cost-effective manner.

- Analysis of allergens in foods: However, much still needs to be done to improve the quality of analytical methodology being used to verify levels of allergens in foods. Through the iFAAM activities it has become evident that whilst current immunoassay test kits are effective at detection of allergens at low levels in foods, they struggle to quantify. There also remain issues over reporting units although, through having analysts and those involved in allergen risk management working side-by-side a consensus has emerged over the need for analytical methodologies to report allergens in units of protein and not commodity, since protein is the hazard and candidate reference doses are expressed in protein. Strides have been made towards multi-allergen, multianalyte mass spectrometry methods for detection and quantification of allergens in foods which will contribute an important complimentary method to the immuno-based methods. It has been proven that the methods can be developed for a number of foods in diverse, difficult-to-analyse matrices such as chocolate.

- Dietary interventions for prevention of food allergy: The iFAAM project has also delivered a knowledge base that is needed by public health authorities to develop new guidance with respect to dietary advice to pregnant and breast feeding mothers and with regards infant feeding practices (including weaning). The integrated data sets from UK and German national studies are hosted within the Allerg-e-lab platform and show that application of the new knowledge that, given early introduction of peanut does appear to protect certain individuals from developing peanut allergy later in life. How this can be rolled out in a public health context without compromising important guidelines on breast-feeding. The iFAAM project also addressed the issues of preventing food allergies, by undertaking an assessment of the evidence base which would ultimately support modification of guidelines for maternal diet during pregnancy and infant feeding practices including weaning.

IMPACT ON CONSUMERS: The stakeholder group representing the food allergic patients has been led by patient group iFAAM partners (DAAB, Anaphylaxis Campaign and Anaphylaxis Ireland) who were actively involved in the research into the allergic reactions in the community, activities relating to understanding the best way to communicate risks posed by unintended allergen presence through the application of PAL, as well as providing insights into what constitutes an acceptable risk. With representation on the project executive committee they have been at the heart of iFAAM, providing direction that has ensured the projects activities deliver the intention of reducing the burden of food allergies on the allergic consumer. They have also provided input into the iFAAM ethical committee. The patient organisations have also worked with the wider patient organisation community to ensure the iFAAM goals and activities have been disseminated and communicated to the two major international networks of patient organisations:

a) The Patient Organisation Committee of EAACI
b) The International Food Allergy and Anaphylaxis Alliance (iFAAA), a global network of patient organisations working in the field of food allergy.

In this way iFAAM activities have gained input from patient organisations from 25 countries across the EU, North- and South America, Australia, Asia and Africa.

Every aspect and result of the project that has given better insight into how to prevent or manage food allergies in daily life impacts on patients with food allergies, their families and those around them. Specific
impacts which have been realised include:

• Managing severe reactions: A standardized approach to classify allergic reactions using the severity scores developed in Module 2 will help allergic patients by providing consistent messages from healthcare professionals and thus being able to better identify, who is at risk of severe allergic reactions. Since management of food allergies is highly dependent on accurate diagnosis this will improve the possibility to generate personalised management plans for patients with food allergies, including dietary advice and provision if appropriate rescue medication.

• Precautionary allergen labeling: More transparent, uniform and meaningful PAL as result of evidence based risk assessment is a major achievement for consumers with food allergies. The iFAAM patient organizations have also developed a consensus that simple to understand phrases, such as “may contain” were preferable. However, allergic consumers wished to have an understanding of the risk assessment process explained that leads to the use of precautionary allergen labels. With regards easing the burden of existing food allergies on the allergic consumers, the iFAAM Tier 1 and Tier 2 risk assessment approaches and associated tool box provide a cohesive, evidence-based approach to managing allergens in foods which have previously been lacking. The application of these tools in future will make the use of precautionary allergen labels much more transparent and thereby ease the burden placed on allergic consumers when shopping or eating out of the home and help them to understanding the risks posed by unintended allergens. Data gaps, such as confirmation of proposed reference doses by single dose challenges, should be filled and analytical methods for allergen detection and quantification should be further improved. Achieving both these goals will contribute to delivering “safer” foods for consumers with food allergies.

• AlleREACT: The roll-out of a tool, in a format that can be readily adopted by the patient organisations beyond iFAAM, has provided them with a tried-and-tested means of collecting important data on reactions experienced by their members. This will support them in their advocacy for change and has the potential to be developed into a way of assessing the impact of any interventions designed to reduce the burden of allergic reactions in the community.

HEALTH CARE PRACTITIONERS: The project has embedded clinical research involving health care professionals (clinicians, dieticians, nutritionists and psychologists). This involvement has enabled health care professionals to gain a deeper understanding of how allergens are managed in the food industry and the issues the industry faces in managing allergens in foods. This knowledge has been disseminated more widely, in particular through the world leading professional association dealing with food allergy, the European Academy of Allergy and Clinical Immunology (EAACI). In addition to publications in the peer reviewed literature (both published and planned), the impact of the iFAAM results have been disseminated into the clinical community through talks and posters at congresses, meetings and training schools organised by EAACI. iFAAM partners have also contributed to the development of an EAACI initiative on harmonisation of severity scoring. Going beyond the project, further dissemination will be facilitated through two task forces of the Food Allergy interest group of EAACI which involve iFAAM partners including one on thresholds led by MCRI (also involving the Charité, SERMAS, UZH, USOU, KCL, UNIMAN, Imperial, UCC) and a second on food processing (led by UNIMAN with input from FBR-DLO, INRA-CRJJ, Charite, KCL and UZH). These activities will ensure the outputs of the re consequence they have been able to and hence give patients advice as to what precautionary allergen labels mean for them in managing avoidance of their problem food;

IMPACT ON THE EUROPEAN FOOD INDUSTRY AND THE INNOVATION UNION INITIATIVE.

With almost one third of the project partners being business-based organisations, iFAAM has intrinsically
had a culture of taking research results and delivering them directly into the user community. Technology transfer has taken place between partners, ranging from knowledge and tools, supporting improved food allergen management in large, multi-national companies, to supporting small business providing consultancy to small food manufacturers (especially SME’s) and transfer of analytical technology from academic research institutes to test kit manufacturers to enable a broadening of their product portfolio. The same priorities exist for the food industry and for food allergic consumers – specifically that well defined reference doses for food allergens are needed and that the risk assessment approach for food allergen management is accepted as the approach of choice by all stakeholders, including regulatory authorities.

iFAAM has generate new knowledge and an effective tool box to equip the food industry (including the SME sector) to deliver foods that are safe for allergic consumers whilst remaining competitive and able to develop new and more innovative products whilst not compromising on allergenic safety. The project has also supported businesses that exist to service the food industry through provision of analytical services, risk consulting and training. New antibody-based and mass spectrometry methods have been developed for analysis of multiple allergens in food. By using food ingredients and real food matrices for their development and validation analytical readouts can be more readily interpreted with regards the risks posed by levels of allergens returned by the methods.

Embedding the development of these tools within a risk assessment framework also helps ensure appropriate testing regimes are employed and that methods have sufficient sensitivity to deliver meaningful results regarding unintended allergen presence at or around reference dose levels. To help the food industry interact more effectively with service providers best-practice guides have been developed to support the food industry in selecting relevant tests, sampling foods in an appropriate manner as well as understanding and interpreting test results. More efficient and effective food allergen management is important to enabling the European food industry to become a Low carbon and resource efficient industry by reducing waste and finding sustainable means of implementing food allergen hazard management, through effective hazard control procedures and cleaning strategies.

OPEN SCIENCE, HEALTH INFORMATICS AND BIG DATA FOR FOOD ALLERGY

The iFAAM partnership has contributed to realisation of the EC vision for “Open Science” in a number of ways including seeking to ensure publications from the project are open access and where this is not possible, author accepted manuscripts are made available through Institutional repositories.

Using ‘big data’ to advance the health and care of patients and the public is a major priority for health research in the 21st Century. As a consequence there is an increasing emphasis on data curation and maintenance to support data integration and reanalysis, especially for large international studies such as EuroPrevall and iFAAM and especially when the research is publicly funded. Through the development of the Allerg-e-lab platform, iFAAM has developed a unique resource which will last long into the future. This will facilitate further data analysis and studies on the cohorts developed to answer questions relating to mechanisms involved in development and prevention of food allergies, biomarkers for severity and the role of specific micronutrients in early life that may play a role in determining susceptibility to allergies later in life. The Allerg-e-lab platform has been developed by the health informatics centre at UNIMAN, which is part of the Farr Institute, a UK-wide research collaboration funded by the UK Medical research Council, the Farr Institute seeks to enable new datasets and developing new infrastructure, methodology, technologies, standards and best practices. Through working with Farr, the iFAAM Allerg-e-lab provides a means for the safe and trusted sharing of data which is linked to other users of the same platform technology. This
includes the STELAR consortium of UK asthma birth cohorts (involving iFAAM partners UNIMAN, Imperial and USOU) and the US National Institute of Health funded CREW consortium data, representing the US asthma birth cohorts. Moving forward the iFAAM partnership will be explore wider collaborations across Europe and beyond to support the further development of the data sets, the platforms that allow their effective curation, harmonisation and analysis. In addition the source code underpinning the AlleRiC on line reactions in the community study data collection interface will be made open source. A crucial aspect in the future will be to use the data sets already developed to help train the next generation of eHealth informaticians by bringing together mathematicians, computer scientists, clinical researchers and basic scientists and help support the development of this cutting edge e-Health research within the European Research Area for which there a skills gap. To that end a health informatics training workshop is proposed to support training Allerg-e-lab partners in the use of the platform as part of the post-project development of the platform.

The Allerg-e-lab has cut across all the project activities with in-clinic data collection facilities and tools that have allowed integration of historic EuroPrevall data sets, along with data sets from the randomised controlled trials aimed at studying the effect of early introduction of allergenic foods into the diets of infants and young children EAT and LEAP. A key aspect of the import of data sets has been issues dealing with the governance of the data. To that end an agreement has been developed in discussion with the relevant iFAAM partners and partners from the EuroPrevall project not involved in iFAAM. A concept of a club in which the members bring their data and can make collective decisions about future use has been developed and agreed. This agreement underpins the governance processes that have been established to manage Allerg-e-lab from user accounts to dataset import, dataset access and data entry. A metadata registry service is being implemented to provide improved support for the collaborative development of the data dictionary and study level metadata.

Lastly the activities developing mass spectrometry methods for determination of allergens in foods have generated large quantities of mass spectral data for allergenic food ingredients (skimmed milk powder, pasteurised egg white powder, defatted roasted peanut flour, hazelnut flour and walnut flour). These data will be deposited in on-line repositories, such as PRIDE (PRoteomics IDEntifications (PRIDE) database) as the reports associated with the development of MS-based methods for allergen detection are published in the peer reviewed literature.

REALISING THE EUROPEAN RESEARCH AREA
The iFAAM project has built on research excellence in Europe in relation to allergic disease, and food allergy in particular, which has spanned more than five framework programmes. The power of this research is a result of the cohesion brought by EU funding, which is also acting as a hub to draw in and capitalise on other projects funded at a national level. In this way iFAAM has helped to prevent fragmentation and hence helped build cohesive evidence base to allow effective management of food allergy across the European Union, which takes account of the cultural and climatic diversity which is a European strength. Such an effort is, inevitably transdisciplinary and a notable achievement of iFAAM has been the integration of health data science into food allergy and the use of mathematical modeling approaches to understand and characterise foods allergies in a way which has not been possible before. The cohesive data sets integrated within Allerg-e-lab make a vital contribution to this activity, both within the project life-time and beyond. It has also brought together patient organisations, who have been actively involved in the research, together with clinicians, mathematicians and computer scientists, as well as analytical and food sciences. None of this activity would have been realised without a European approach developed over many years and which has helped make Europe a world leader in all aspect of allergy.
research. Tangible examples of the added value and impact this will derive are as follows:

EuroPrevall (FOOD-CT- 2005-514000): This integrated project with 62 partners from more than 17 European countries, India, China and Russia together with collaborators in N America and Australia sought to deliver the information and tools necessary for policy makers, regulators and the food industry to effectively manage food allergies across Europe and hence deliver an improved quality of life to food allergic consumers. The EuroPrevall birth cohort also collected unique data on maternal diet during pregnancy and during weaning. Eighteen EuroPrevall partners contributed to iFAAM (AMC, Charité, BOKU, UNILEVER, SERMAS, PEI, INRA- CRJJ, DLO- FSB, USOU, MUL, UOA, LUH, UZH, ACP, UCC, DTU, NESTEC and Imperial) and building on this core expertise and knowledge of EuroPrevall outputs were built on by iFAAM activities to realize the following:

- EuroPrevall data sets contributed significantly to data integrated into the Allerg-e-lab health informatics platform. It provided the core concept of common tools for diagnosis and centralised databases for collection of data in a unified manner to facilitate subsequent data analysis. Clinical record forms and quality of life questionnaires have reused and adapted those developed in EuroPrevall for all clinical studies undertaken.

- Approaches to manufacturing and dose verification for oral food challenges developed in EuroPrevall have been refined beyond the EuroPrevall dessert matrix extended to include the iFAAM cookie matrix.

- Data sets developed in EuroPrevall have been enlarged with:
  - A follow-up of the EuroPrevall birth cohort to collect data on patterns of food allergy at school age enabling the relationship between dietary factors and allergy outcomes later in life to be followed.
  - Integrate data on intervention studies using proton pump inhibitors and a comparison of the food matrix in oral food challenge studies.
  - Data on single dose challenges used to verify potential reference doses for hazelnut, egg and milk.
  - Collect data on reactions in the community

- iFAAM extended biobanking and collection of serological data on patients begun in EuroPrevall and has extended the analytical approach developed in EuroPrevall with serum samples from all patients recruited into iFAAM studies

- Contributed to the enlargement of a database of threshold doses developed by iFAAM partners FARRP and TNO to improve the quality of the evidence base underpinning food allergen management risk models.

MoniQA (Food- 2006-CT-036337): MoniQA began as a network of excellence which has developed into an association which seeks to harmonise global food quality and safety monitoring. In this way the network members seek to add value to the food chain to ensure consumer confidence and ensuring effective and efficient food quality and safety regulations within the EU and worldwide. One way in which it seeks to achieve this is by establishing common methods and standards in food analysis, including allergens. Four iFAAM partners are involved in the MONIQA network, the MONIQA-Association itself, BOKU, JRC-IRMM and EuroFIR. Specific aspects that this network have contributed to are the development of multianalyte methods of analysis of allergens in foods, and have contribute to enlarging the analytical science expertise of MoniQA Association with that of allergen management coming from iFAAM.

EuroFIR (CT- 513944) and EuroFIR Nexus (CT-265967): A network of excellence which has resulted in the formation of an international non-profit association, EuroFIR AISBL, EuroFIR developed an information source regarding food composition, including a European-wide food composition database. In addition to Eurofir, AISBL other iFAAM partners belonging to the EurFIR network are UCC, DTU, ILSI and ANSES. The EuroFIR standardized food information tools have made an important contribution to the recoding of
the EuroPrevall and iFAAM datasets to enable effective interrogation of the nutritional data they contain. This is helping to support the analysis of this information with regards maternal diet during pregnancy and infant feeding practices in relation to allergy outcomes later in life.

In addition to projects funded at a European level, iFAAM has drawn on data and expertise built up obtained through various nationally-funded activities from the UK Food Standards Agency which has shared data, expertise and tools from its research as follows:

- **Birth cohort follow-up**: The UK FSA has funded studies in the Baseline cohort (IE; FSA project code T0706) and contributed the challenge data to be made available, allowing a much more effective harmonisation of data between the EuroPrevall and Baseline cohorts when they were followed up at school age.

- **Investigations into early introduction of allergenic foods in preventing food allergy**: The Enquiring About Tolerance (EAT; FSA project code: T07051) and Learning Early About Peanut Allergy (LEAP; FSA project code T07049) studies have made a significant contribution to the project in terms of data and biological samples.

- **Impact of extrinsic factors on reactivity to peanut**: TRACE (FSA project code: T07068) aims to establish whether exercise and sleep deprivation modulate the threshold of reactivity and effect the clinical severity of allergic reactions in a representative group of the peanut allergic population. The FSA enabled sharing of clinical protocols developed as part of the study, together with data on up to 20 patients challenged with peanut in the EuroPrevall chocolate dessert challenge matrix at Imperial to support iFAAM. They also helped to support the workshop on severity scoring by funding TRACE investigators to attend an iFAAM workshop on the subject.

- They have also contributed actively to all iFAAM workshops and meetings, being part of the risk assessors and regulators stakeholder group and hosting a workshop on infant diet and the development of allergy at their office in London UK.

Other research that has been funded at a National level on which iFAAM has built includes the Australian National Institute for Health and Medical Research Council which has funded the RCTS on early introduction of allergenic foods into the infant diet with the Solids timings for Allergy Research [STAR], Starting Time for Egg Protein [STEP ] and Beating Egg Allergy [BEAT] projects and the German nationally funded projects Hens Egg Allergy Prevention [HEAP] and Preventing Peanut Allergy in Atopic Dermatitis [PEAAD]. The French national funding agency, ANR also contributed data and tools through the Mirabel project. FARRP and TNO have also brought their unique data sets of threshold dose distributions collected through studies undertaken by the FARRP consortium and ILSI-Europe funded studies with other data generated notable through the EuroPrevall project and subsequently analysed using National funding (UNIMAN). The EuroPrevall data sets will be made available via the Allerg-e-lab platform. When combined with other data from FARRP and ILSI this provides the largest set of data on threshold doses in the world.

**CONTRIBUTION TO STANDARDS**

The contribution of iFAAM to standardisation spans a number of domains and will be disseminated to the wider community through the activities of Module 5 and the interactions with the various stakeholder groups as follows:

Harmonisation of clinical protocols and diagnosis of food allergies: iFAAM has built on activities undertaken in EuroPrevall, other EU-funded initiatives, such as Galen, and those led by EAACI, to harmonise the manner in which patients are diagnosed. This relates to common clinical history questionnaires, diagnostic protocols (especially DBPCFC) and collection of symptoms. The development
of a harmonised approach to severity scoring and especially the iFAAM ordinal FASS has built common definitions and application of in scoring symptoms, especially those observed during food challenges, as well as definitions of severity, including anaphylaxis. The project partnership will seek to make the clinical record forms publicly available via the Allerg-e-lab IT platform. In addition the project partnership explore the opportunity to make a submission to the EU-funded CHRODIS platform CHRODIS Platform which is developing an up-to-date repository of the good practices identified on the prevention and care of chronic disease. (http://chrodis.eu/about-us/)

Analysis of allergens in foods: Although there are a lot of immune-analytical methods as commercial kits available for the detection of the different food allergens with respective validation data there are no measurement methods as International Standards until now. Harmonisation of reporting units has been a key goal of the community. Working with the MFAN network of companies and the iFAAM project partnership a consensus has been reached that reporting of analytical measurements needs to be in protein rather than commodity. This will make it easier for food business operators to integrate analytical measurements of unintended allergens within a risk assessment context and constitutes best practice in the protein represents the allergenic hazard. Through undertaking a ring trial for ELISA test kits and the newly developed targeted mass spectrometry methods for analysis of peanut also helps improve the performance of analytical laboratories as well as helping to identify best practice and allow platforms comparisons to be made which enhances competitiveness between test kit manufacturers and MS vendors. The iFAAM MS methods could form the basis for development of international standards and accepted methods, including explicit MS measurement parameters, protein and peptide standards and approaches for further method validation. In addition outputs of iFAAM will support the development of clinically-validated reference and quality control materials which are much needed by the analytical community to support inter-laboratory trials, both to improve the quality of analysis undertaken by different laboratories but also to support assessment of methods. A tangible outcome is that the peanut flour used in the ring trial is now being supplied as a standard through LGC Standards to help support harmonisation of reporting units for allergen analysis.

Food allergen management in the food industry: Defined and validated dose distribution curves for regulated allergens will contribute to the development of harmonised reference values for those allergens, which will serve as an essential foundation for operational allergen management thresholds for implementation by public health agencies and the food industry. Improved understanding of processing unit operations and contamination scenarios will contribute to development of good practice guidance for allergen management again using the reference values as a foundation. The tools developed in the project will provide harmonised, standardised means of undertaking food allergen management in the food industry; a particular emphasis will be on providing SME food producers with usable tools and equip the sector providing training with harmonised, common approaches. The project partnership has also driven harmonisation of reporting units for allergen analysis

Harmonisation has been highlighted as key to improving industrial competitiveness in Europe and this is especially important with regards food safety issues, which represent an important cost for food manufacturers. Through the activities of iFAAM, the harmonised allergen management plans needed by the industry have been developed, which also take explicit account of the needs of SMEs (which represent a crucial community in the food sector in particular). Furthermore the data and tools, in particular the clinically validated risk models, will develop evidence and approaches that will allow action levels for allergens to be set in the coming years. The involvement of project partners from the USA and Australia, together with the global nature of the ILSI, MoniQA Association and EuroFIR networks as well as
organisations such Food Drink Europe, will also help support harmonisation and dissemination of this approach across the world in the future.

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Related documents

-final1-ifaam-final-report-ver-6.pdf

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