



Approaches to risk assessment in food allergy: Report from a workshop “developing a framework for assessing the risk from allergenic foods”[☆]

C.B. Madsen^{a,*}, S. Hattersley^b, J. Buck^b, S.M. Gendel^c, G.F. Houben^d, J.O'B. Hourihane^e, A. Mackie^f, E.N.C. Mills^f, P. Nørhede^a, S.L. Taylor^g, R.W.R. Crevel^h

^a Department of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark, Research Leader, 19 Mørkhøj Bygade, DK-2860 Søborg, Denmark

^b Food Standards Agency, London, UK

^c Food and Drug Administration, USA

^d TNO, Quality of Life, Zeist, The Netherlands

^e Department of Paediatrics and Child Health, University College Cork, Ireland

^f Institute of Food Research, Norwich, UK

^g Department of Food Science and Technology, University of Nebraska, Lincoln, Nebraska, USA

^h Safety and Environmental Assurance Centre, Unilever Colworth, Sharnbrook, UK

ARTICLE INFO

Article history:

Received 1 July 2008

Accepted 2 December 2008

Keywords:

Risk assessment

Food allergy

Uncertainty factors

Margin of Exposure

Probabilistic

ABSTRACT

A workshop was organised to investigate whether risk assessment strategies and methodologies used in classical/conventional toxicology may be used for risk assessment of allergenic foods, to discuss the advantages and limitations of different approaches and to determine the research needed to move the area forward. Three possible approaches to safety assessment and risk assessment for allergenic foods were presented and discussed: safety assessment using NOAEL/LOAEL and uncertainty factors, safety assessment using Benchmark Dose and Margin of Exposure (MoE), and risk assessment using probabilistic models.

The workshop concluded that all the three approaches to safety and risk assessment of allergenic foods should continue to be considered. A particular strength of the MoE and probabilistic approaches is that they do not rely on low-dose extrapolations with its inherent issues. Probabilistic modelling is considered to be the most promising approach for use in population risk assessment (which is a particular focus for risk managers). For all approaches, further improvement of input data is desirable, particularly data on consumption patterns/food choices in food allergic consumers, data on minimum eliciting doses and data that can be used to evaluate whether the whole population at risk has been modelled accurately. Specific research topics were identified.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

It is well recognised that allergenic foods represent a significant health hazard for those who have an allergy or intolerance to specific foods. However, this hazard remains poorly characterised although hazard identification and characterisation are vital components of the risk assessment process. Increasing amounts of data

are now becoming available with which to better characterise the hazard and therefore assess the risk from food allergens.

Food allergens present risks to sensitive consumers both when they are used as ingredients in food products and through cross-contamination of foods with allergenic components that are not used as ingredients. Production of food without cross-contamination from allergenic ingredients that are commonly used in manufacturing such as milk, wheat, peanut and egg, is complicated and often difficult to achieve. Many food products could not be produced economically if manufacturers were required to guarantee total absence of specific allergenic constituents.

Current European, US and other legislation on allergenic food ingredients define which allergenic foods must always be declared on a product label. Except for Switzerland, this only applies to ingredients deliberately added to food according to recipe. This legislation does not set any specific thresholds for labelling of these allergenic foods. Regulatory authorities have generally concluded

Abbreviations: BMD, Benchmark Dose; DBPCFC, double-blind placebo-controlled food challenge; LOAEL, Lowest Observed Adverse Effect Level; MED, minimum eliciting dose; MoE, Margin of Exposure; MoS, margin of safety; NOAEL, No Observed Adverse Effect Level.

[☆] The opinions and conclusions expressed in this article are solely the views of the author(s) and do not necessarily reflect those of the US Food and Drug Administration.

* Corresponding author. Tel.: +45 72347563; fax: +45 72347001.

E-mail address: charm@food.dtu.dk (C.B. Madsen).

that data were inadequate to define safe thresholds for food allergens, although they accepted that such thresholds do exist (EFSA, 2004, Threshold Working Group, 2008). Current EU (and most other) legislation also has not addressed directly the issue of allergen cross contamination. Whilst there is some voluntary guidance which includes qualitative advice for industry on how to assess and manage risk from allergenic foods (Food Standard Agency, 2006), there is currently no advice from regulatory bodies or compliance authorities on levels of allergen cross contamination above which advisory labelling (such as 'May Contain Nuts') should be used.

There is therefore a lack of guidance at the present time for those involved in allergen risk management. Risk managers would like to be able to set so-called management or regulatory thresholds for allergenic foods, which would represent a tolerable level of risk to the population at risk. This approach recognises that zero risk is unachievable, and is distinct from the definition or establishment of a toxicological threshold, which is more commonly taken to mean a dose at, or below which, a response is not seen in an experimental setting (Kroes et al., 2000). Definition of acceptable or tolerable levels of risk with regard to allergenic foods requires input from all stakeholders with an interest in food allergy.

This current absence of defined thresholds has consequences for risk managers, who have to decide what level of allergenic food in a given product constitutes a health risk and therefore requires action to manage and/or communicate the risk. It is also a problem for enforcement authorities, who have to decide whether or not to take action against products that contain particular levels of allergenic material. Lastly, there are consequences for the consumer, if the lack of defined thresholds leads to a proliferation of allergen precautionary labelling and unnecessary restrictions in choice for the most cautious, risk-averse consumers. In contrast, there is an evidence that even the current level of precautionary labelling has led a significant proportion of allergic consumers to ignore such warnings, as they are so widespread, thereby putting themselves at risk (Food Standards Agency, 2002; Hefle et al., 2007).

For regulators to be able to set risk-based thresholds for allergenic foods, a clear scientific consensus is required on how to assess risk to the allergic population from particular levels of allergenic constituents in food products, and also on the appropriate level of protection. Whilst there are well-accepted methods for setting regulatory limits based on the results of toxicological studies, that take account of inter-individual and inter-species variations, and that protect the most vulnerable subgroups of the population, no such consensus exists for allergen risk assessment.

In food allergy, risk management decisions are often required before either sufficient data are available or methods to analyse the data are developed. Questions have even been raised regarding the appropriateness of the classical risk assessment approach to allergenic foods (Kroes et al., 2000). Recent work has indicated that it might be possible to develop more efficacious new approaches (Crevel et al., 2007). There is an urgent need for further development of new methods as well as international agreement on how to apply these methods to risk assessment for allergenic foods.

In toxicology, risk assessment is based on toxicity data generated by toxicologists in an appropriate range of studies conducted under carefully controlled conditions, almost always in one or more animal species. In food allergy, data on population dose distributions are collected directly in humans (via food challenges performed in allergy clinics) as no suitable animal models exist to characterise the risk to man from food allergens. Thus, to perform risk assessments for allergenic foods, data generated by clinicians are mandatory. For ethical and practical reasons, the data available on allergenic foods will always be a compromise, but toxicologists and clinicians need to communicate and agree in order to

generate data, which meet the needs of food challenge study participants, clinicians and risk assessors. Another distinction is that the population we wish to protect is not theoretical subjects that may or may not get a disease in the future but people with names and faces and families, whose lives may be endangered if the risk is not properly managed and communicated.

Against this background, the EU project EuroPrevall, together with the UK Food Standards Agency and ILSI Europe convened a multidisciplinary International Workshop in May 2007 with invitees from several Governments, the European Food Safety Authority (EFSA), Universities and Research Institutes, Allergy Clinics, Industry and Patient Organisations. The purpose of the workshop was to investigate whether risk assessment strategies and methodologies used in classical/conventional toxicology may be used for risk assessment of allergenic foods, to discuss the advantages and limitations of the different approaches and to determine the research needed to move the area forward.

The workshop considered three possible approaches to safety assessment and risk assessment for allergenic foods, which are summarised and discussed below. The recommendations and conclusions resulting from the workshop as well as the research needs that were identified are also presented.

2. Safety assessment and risk assessment

Safety assessment and risk assessment are part of the risk analysis concept, which also includes risk management and risk communication. These elements are separate tasks often performed by different players, but should be part of an interactive and iterative process (Larsen, 2006). Ideally, the safety assessment or risk assessment of allergenic foods is a purely scientific process that utilizes expertise in food allergy, toxicology and nutrition (for the intake assessment).

Safety assessments and risk assessments have been widely used to establish regulatory thresholds for chemical contaminants and additives in foods. The basic principles underlying these approaches have been described by the World Health Organization and associated organisations (FAO/WHO, 1997; Codex Alimentarius Commission, 2003) and the application of these approaches to food allergens was discussed in the FDA Threshold Working Group Report (2006).

A safety assessment for a food additive or contaminant results in the calculation of an Acceptable Daily Intake (ADI; additives) or a tolerable daily or weekly intake (contaminants). The acceptable level of intake is based on 'no effect levels' from animal experiments and, where possible, human data. Uncertainty factors are used to derive from these animal data the amount that allows a daily intake over a lifetime without appreciable health risk (WHO, 1987). No quantitative estimate of risk is calculated.

For chemicals where a 'no effect level' cannot be established, the risk (for example, of getting cancer at a certain contamination level) may be calculated using mathematical modelling of experimental data. The mathematical modelling uses low-dose extrapolation beyond the experimental results of animal studies and the output is heavily influenced by the model used. To avoid the uncertainties associated with such estimates the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and European Food Safety Authority (EFSA) have used the Benchmark Dose concept to estimate a reference point to be used to calculate a Margin of Exposure (MoE). This does not lead to a quantitative risk assessment, but comparing the MoEs for different carcinogens and different exposure scenarios allows risk managers to prioritise risk management actions (Larsen, 2006).

Safety and especially risk assessment of food allergens differ from most other assessments of food-borne chemical hazards

because only a small part of the population is at risk. In addition, the allergenic food that may be lethal to consume for the food allergic person is often an important nutrient for the rest of the population.

The approaches described below are applicable to food containing allergens by carryover in an even distribution. Workshop participants agreed that these assessments approaches could not easily be used to assess the risk from sporadic contamination with particles such as whole seeds or pieces of nuts, for which a different approach will be required.

Current legislation on allergen declaration assumes that food allergens relevant for risk assessment are stable and that their potency is neither enhanced nor reduced during food processing. The only consideration is therefore how much is present. Future research may prove this approach to be too simplistic.

The three approaches presented below should be seen as attempts to demonstrate the proof of principle and not as ultimate safety and risk assessments.

2.1. Safety assessment in food allergy using threshold (NOAEL) and uncertainty factors

The application of this approach depends on the use of data from controlled studies that identify a No Observed Adverse Effect Level (NOAEL) and a Lowest Observed Adverse Effect Level (LOAEL) for a particular substance and a defined response using either animal models or human clinical data. An Acceptable Daily Intake (ADI) level or other health based guidance values can be determined by applying a “safety factor” or “uncertainty factor” to the NOAEL or LOAEL to account for the uncertainties associated with extrapolation from animal data to humans, variation within the human population, and other uncertainties specific to the substance or data set being considered. For allergenic foods, extrapolation from animal data is not relevant. However, there are uncertainties introduced by factors such as the size of the test populations in clinical studies, how well these test populations represent the general food allergic population, and how preparation and presentation of the test material affect the outcome of challenge tests (measured allergenic potency).

The FDA Threshold Working Group outlined one example of how this approach might be used for food allergens (Threshold Working Group, 2008). Although not intended to represent a full safety assessment, the example using peanut highlighted the major steps in the safety assessment process. The group reviewed the available literature to identify clinical studies that reported LOAELs and NOAELs. They found that, although a number of studies reported apparent LOAELs, NOAELs could not be determined in these studies because some patients responded to the lowest dose tested. In that literature review, two studies that did report NOAELs were identified; Hourihane et al. (1997) reported a NOAEL of

50 µg peanut protein per person and Wensing et al. (2002) reported a NOAEL of 30 µg peanut protein per person based on subjective symptoms. The group also discussed factors to be considered in determining the appropriate uncertainty factor to use with these data. These factors included the small size of the test populations, exclusion of potentially more sensitive individuals who have previously experienced severe reactions or anaphylaxis, variation in preparation and masking of the test material, differences in dose progression, and differences in describing and recording the signs and symptoms. In this case, it was not necessary to consider uncertainty associated with extrapolation from animal models to the human population. The group suggested that a 10-fold uncertainty factor might be appropriate to account for variation in the sensitive population beyond that seen in the small number of individuals in these clinical tests. An additional 10-fold factor might be appropriate to account for exclusion of the most sensitive individuals and children from these studies (Table 1). If these factors were to be used in an overall uncertainty factor of 100×, along with a NOAEL per person of 30 µg peanut protein, the potential regulatory threshold would be 0.3 µg of peanut protein per person. It might be appropriate to include an additional uncertainty factor in an analysis that included consideration of studies reporting only LOAELs.

2.2. Strengths

- This is a widely accepted approach that has been successfully applied to a large number of food additives and chemical contaminants.
- This is an inherently conservative approach because it is based on the response of the most sensitive individuals in the population of interest.
- This approach can be used in the absence of data on exposure or consumption. This is particularly important for food allergens because sensitive consumers practise avoidance diets and most exposure for these consumers are inadvertent and therefore difficult to measure or characterise systematically.
- It makes relatively few assumptions.

2.3. Weaknesses

- The data analysis uses a single data point from a single study, without any regard to the shape of the distribution.
- Variability exists between publications in the way that reactions are characterised and reported. For example, many studies only report objective responses. Therefore, it is not possible to evaluate the potential for using LOAELs and NOAELs based on subjective responses in the safety assessment-based approach.

Table 1

Example of uncertainty factors for the safety assessment-based approach using peanuts.

Description	Uncertainty factor	Justification
Intraspecies difference ^a	10	Standard factor for intraspecies variability
Estimation of NOAEL ^b	Not applicable	Two studies were identified that report NOAELs
Sensitive population ^c	10	Used to account for additional margin of protection for more susceptible populations not included in clinical trials
Overall uncertainty factor for peanuts = 100		

Example of uncertainty factors and their justification.

^a This includes both between- and within-individual variability.

^b This includes both a factor for converting the LOAEL to a NOAEL and an additional factor for the uncertainty associated with that conversion. In this example for peanuts, there are data on both subjective and objective NOAELs and LOAELs. If the NOAEL values are used, the uncertainty factor is 1-fold (i.e., not applicable). If the LOAELs had been used, this value would have been higher. If subjective symptoms observed at lower levels are used, a different uncertainty factor may be considered.

^c This includes uncertainty associated with an additional margin of protection to account for the potential severity of reaction (e.g., lethality) for the highly sensitive subpopulation.

- In many published studies some individuals are reported to have reacted to the initial dose tested. Therefore, the data in these publications cannot be used to determine a NOAEL or whether the initial dose is the actual Lowest Effect Level.
- The number of individuals tested in a clinical study is often relatively small because the procedure is onerous and it can be very difficult to find suitable individuals. In addition, individuals who have experienced severe reactions in the past, or who have other markers of high sensitivity, have often been excluded from clinical trials in the past affecting the data available for use in risk assessments.
- There is often little information in studies on how the participants relate to the overall allergic population.
- General application of this approach could result in regulatory thresholds which are below those that can be reasonably attained in general food manufacturing, and indeed below those that could be reliably measured with current assays.

2.4. Safety assessment in food allergy using Benchmark Dose and Margin of Exposure (MoE)

In addition to the classical NOAEL safety assessment approach there is another toxicological safety assessment model, which uses the concept of Benchmark Dose (BMD) and permits the calculation of a Margin of Exposure (MoE). The BMD model was developed by Crump (1984) to make better use of experimental data than possible when using the NOAEL alone. The method can be used both for incidence (quantal data) and for continuous data. In its original application to chemical carcinogens, data from animal studies are used to fit a curve using different mathematical models. The Benchmark Dose may then be defined as the dose that induces cancer in 10% of the animals. The dose used for further calculations (point of departure) is usually the lower 95%-confidence interval of the Benchmark Dose. This is called the Benchmark Dose lower limit (BMDL). It is important that the Benchmark Dose lies within (or very close to) the experimental data points. This makes the method less sensitive to the choice of mathematical model. The BMDL can be divided by the estimated intake of the chemical in the population resulting in a Margin of Exposure. The size of the MoE is used to decide whether risk reduction measures should be taken (Larsen, 2006). The Margin of Exposure may also be called the Margin of Safety. In a recent paper, Crevel et al. (2007) discussed the use of distribution models of food allergy challenge data to determine a Benchmark Dose.

An investigation of the feasibility of a Benchmark Dose and Margin of Exposure approach for setting tolerable levels of allergen

contamination in food was undertaken for this workshop using peanut as a model food.

2.5. Benchmark Dose – peanut

Data from three studies in children specifying individual challenges data were available May (1976), Bock et al. (1978) and Flinterman et al. (2006). From these studies, the lowest challenge doses (minimum eliciting dose) giving objective symptoms in each subject was used for the modelling. The data from the top dose open challenge in the Flinterman study was also used. The challenge material dose was recalculated to the equivalent amount of total peanut protein. The Weibull mathematical model gave a better curve fit at the lower end of the dose range tested and the most conservative estimate and was therefore chosen for further calculation. Fig. 1 shows the data from 41 positive challenges using the Weibull model for the curve fit. The Benchmark Dose Lower confidence limit for a 10% incidence (BMDL₁₀) was calculated based on the bootstrap method. The bootstrap results are based on $N = 10,000$ samplings from the original data set, that is, so-called non-parametric bootstrap.

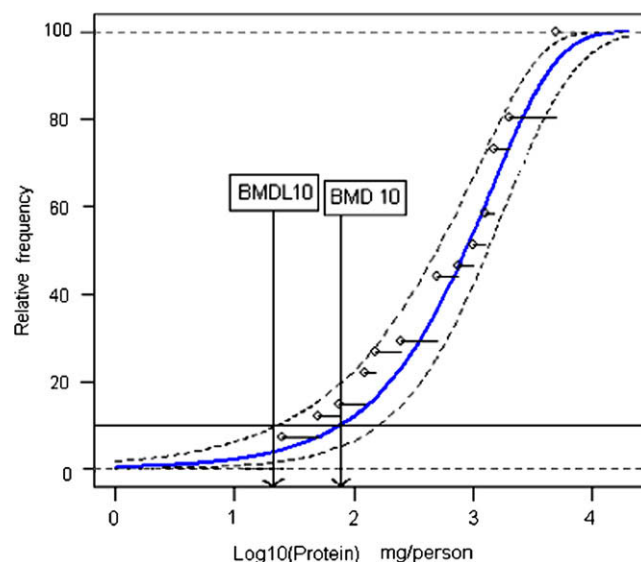


Fig. 1. Challenge data (minimum eliciting dose) from three studies of peanut allergy in children. The curve is calculated using the Weibull model. The BMD₁₀ is the dose that gives reaction in 10% of the tested population. The BMDL₁₀ is the dose corresponding to the lower 95%-confidence interval.

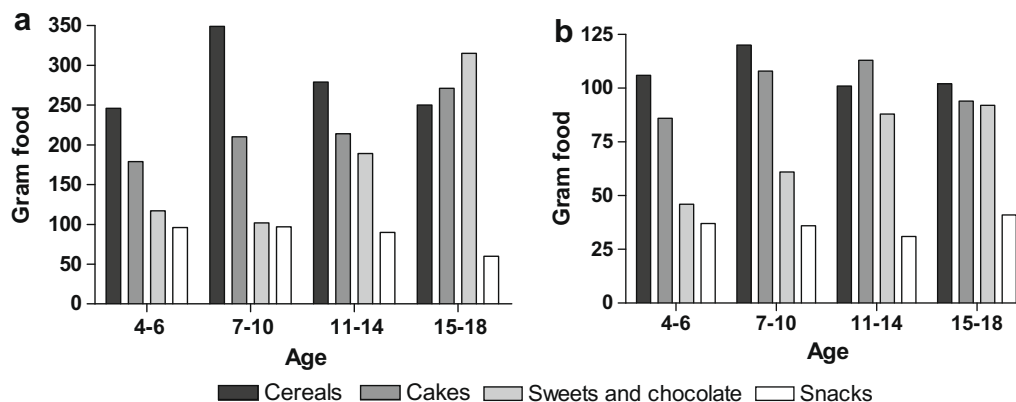


Fig. 2. Food consumption in children from the Danish National Food Survey: (a) maximal daily intake of foods with potential peanut contamination and (b) 95th percentile of daily intake of foods with potential peanut contamination.

The BMD estimated to give a reaction in 10% of the peanut allergic population was 87 mg peanut protein.

The lower 95%-confidence interval of this figure, i.e. the (BMDL₁₀) was 31.4 mg peanut protein per person.

2.6. Food intake and exposure

To calculate the intake of peanut protein from potentially contaminated food, the foods most likely to be contaminated were considered to be breakfast cereals, cakes, sweets and chocolate and snacks.

The intake data used were from the Danish National Food Survey 2000–2004 (Fagt et al., 2007). In toxicological risk assessment the mean daily intake is normally used. This is not relevant in IgE-mediated food allergy where the acute exposure during a meal/eating occasion is the relevant measure of exposure. However, because of the lack of detailed data we were limited to looking at the exposure during an entire day and not on separate eating occasions.

Two different intake scenarios were used. Fig. 2a shows the maximum intake of the foods that may be contaminated with peanut as an example of extreme consumption. Fig. 2b shows the 95th percentile of the intake in the population studied. This figure also includes subjects that do not eat the food. Of the four food groups, breakfast cereals are consumed in the largest quantity and are therefore chosen as the worst case. In addition data from the age group (7–10 years) with the highest consumption of these foods is used. These give figures of 349 g of breakfast cereal per person using maximum intake (Fig. 2a) and 120 g using 95th percentile (Fig. 2b). The consumption data for the four groups are not added, as the combined consumption is not expected to exceed the single consumption, e.g. breakfast cereals and cakes are not eaten at the same occasion.

2.7. Exposure to peanut

2.7.1. Assumption

Breakfast cereals are contaminated with 10 mg peanut/kg food

Exposure based on maximum intake = $349 \text{ g} \times 10 \text{ mg/kg}$
= 3.5 mg peanut per serving,

Exposure based on 95th percentile = $120 \text{ g} \times 10 \text{ mg/kg}$
= 1.2 mg peanut per serving.

2.8. Margin of Exposure

MoE (maximum intake) = $\text{BMDL}_{10}/\text{Exposure} = 31.4/3.5 = 9$,

MoE (95th percentile) = $31.4/1.2 = 26$.

2.9. Conclusion

The MoE provides data to describe the safety or lack of safety of a given level of carryover allergen content and may inform the discussion on setting acceptable levels of contamination: the larger the MoE the less reason for concern. The discussion of the use of and size of uncertainty factors is also relevant in determining the acceptable size of the MoE.

There has been much emphasis on getting and using the best possible food challenge data to determine eliciting doses. The two examples also illustrate that the choice of intake data greatly influences the MoE, here ranging from 9 to 26. It will therefore be important to discuss how intake data should be used in food allergy safety and risk assessment. A conservative approach would be to calculate the intake using the 95th percentile of 'eaters only' calculated on a per meal/eating occasion basis.

2.10. Strengths

- MoE is calculated using the BMDL derived from the distribution of challenge data using a combination of as many challenge data points as possible. This increases the robustness compared to using a single data-point from a single study as in the NOAEL approach and takes account of the shape of the whole dose-distribution, rather than a single dose as point of departure.
- This approach favours good study design (Crump, 1984).
- Once a BMDL for a specific food is established it is easy, in risk management situations, to calculate the MoE for different intake and contamination situations or scenarios.
- It makes decision making potentially more consistent.
- The method is transparent.

2.11. Weaknesses

- The MoE approach does not describe the risk quantitatively, i.e. the likelihood that an incidence will occur.
- The validity of the BMD with regard to the allergic population will depend on how well the study population represents the allergic population.
- It is sensitive to how and which intake data are used.
- Intake data from dietary surveys in the general population may not accurately reflect consumption patterns in the allergic consumer, which could result in over or underestimates of the risk.
- It requires a decision on the desired Margin of Exposure or margin of safety.

3. Risk assessment in food allergy using probabilistic models

3.1. Background

In food allergy risk assessment, a deterministic approach, which uses a worst case scenario (e.g. most reactive individual together with highest intake) will in many cases lead to a conclusion that an allergic reaction cannot be excluded. However, it will not quantify the risk and will often therefore be of limited value to risk managers. An approach aimed at a zero-risk thus leads to a zero-tolerance approach, which can pose serious practical, technical, and financial problems to all stakeholders. To address such dilemmas, insight into actual risks associated with the presence of allergens in food was seen as a key step. TNO therefore developed a quantitative risk assessment approach for allergens in food based on probabilistic principles.

3.2. Probabilistic risk assessment for allergenic foods

The conceptual model has been described in detail by Spanjers-berg et al. (2007). The model calculates the most likely number of allergic reactions that might result from the accidental presence of an allergenic constituent in a food product. This calculation factors in distributions of the minimum eliciting doses (MEDs), together with those associated with variables determining the intake of the allergenic constituent. These include presence and concentration in the affected food, likelihood that an allergic person consumes the food and amount of the food consumed per eating occasion. The concept is illustrated visually in Fig. 3.

In the absence of incidence data, formal validation remains impossible at present. However, sensitivity analyses have been undertaken as part of a collaboration between TNO and the ILSI-Europe Food Allergy Task Force (Kruizinga et al., 2008) involving different scenarios in which the input variables were changed. The sensitivity analyses were performed both on the MED and on the exposure components of the model. We explored the impact

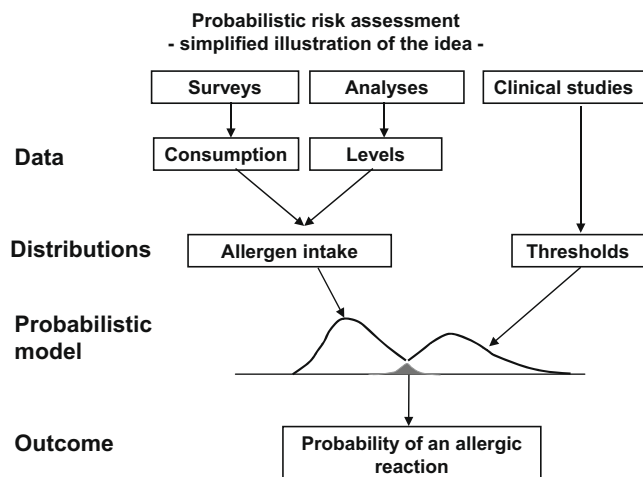


Fig. 3. The probabilistic model estimates the risk of any allergic reaction based on the distribution of food intake and concentration of allergenic food and the distribution of minimum eliciting doses (thresholds) from clinical trials.

of (1) using different statistical model choices for describing the distribution of MEDs, (2) a shift in the distribution of the MED to account for differences in the allergenic potency of different protein sources, (3) the likelihood that an allergic person consumes a certain product, (4) the amount of the food product consumed and (5) a variation in the distribution of the MED associated with the nature of the allergic reaction. Overall the work demonstrated that the location of the distribution of the MED and the proportion of the population consuming a food both have a large influence on the number of allergic reactions using this risk assessment model for allergens. Whilst this might be expected intuitively from the type of data, the model provides quantitative measures to this observation, which can improve the setting of priorities for risk management. Probabilistic modelling can thus help provide an understanding of the major factors which influence the likely frequency of allergic reactions to adventitious protein. This type of analysis can both help to indicate where research to generate data should focus for maximum benefit and enable comparisons between the effectiveness of different risk reduction measures. They revealed that the MED distribution and the frequency of consumption of the affected food were most influential on the risk assessment outcome. In contrast, the statistical model used to fit the MED distribution, the portion size of the affected food and using a more “severe” endpoint (mostly GI-tract effects instead of Oral Allergy Syndrome) as the basis of the MED had relatively minor influence under the conditions examined. The risk assessment outcome thus appears quite insensitive to differences in statistical model choices for describing the distribution of MEDs. This is potentially an important advantage of this probabilistic approach. Development of this work will help to generate a more accurate picture of the potential public health impact of the inadvertent presence of an allergen in food. It also highlights the need for more research, specifically the determination of minimum eliciting doses and an understanding of the food choices of allergic individuals.

Recently, the approach was used in an investigation of the impact of the presence of milk proteins in dark chocolate, following the report of a consumer reaction (Spanjersberg et al., submitted for publication). Analyses revealed the presence of milk proteins in the complaint sample, but also in samples of other batches of the product from the same manufacturer as well as in samples of similar products produced by other manufacturers. The risk associated with the levels of milk proteins measured in those products was estimated using a probabilistic risk assessment model. This model used consumption data from the Dutch 3rd national food

consumption survey. Intake data were based on the amount consumed on each individual meal/eating occasion. Besides information on the amounts of the product consumed, information on the percentages of users was also used (the percentage of users of the product among males is 6.0%, and among females 4.8% per meal/eating occasion). Threshold data for allergic reactions to milk proteins among adults were derived from Lam et al. (2008). Based on these input data, the risk assessment indicated that for the product with the highest contamination level, up to almost 3.5% and 2.5% of the allergic male respectively female population would be at risk for an allergic reaction. Based on a population of about 16.6 million people in the Netherlands and an overall prevalence of milk allergy of 0.3%, these figures correspond to approximately 620 females and 870 males at risk of a reaction if they consume the product. On the basis of this information, the company reassessed its allergen management procedures.

The probabilistic risk assessment methodology can be used to estimate the proportion of an allergic population that may suffer from a reaction due to the presence of a certain level of (undeclared) allergen in a food product. The probabilistic approach can also be used in risk analysis processes, for instance in assessing the potential health benefit of the development and use of more sensitive analytical methodologies or in establishing the analytical sensitivity needed for a certain protection level.

3.3. Strengths

- This approach can quantitatively link information on allergen concentrations in food products to the risk of a reaction, since it uses quantitative data on all the components.
- It makes optimal use of all available information, including dose-distribution and allergen content distribution data.
- It favours good study design.
- It appears to be relatively insensitive to differences in statistical model choices for describing the distribution of minimum eliciting dose.
- No low-dose-extrapolation is needed.
- Safety factors are not needed by definition, but can be incorporated if desired, depending on the goal of the risk assessment or the risk management question to be addressed.

3.4. Weaknesses

- As for other risk or safety assessment approaches, the reliability of the risk assessment outcome depends on the quality of the input data, including in particular the extent to which the sample used to derive the MED distribution is representative of the overall allergic population. It is also sensitive to the use of intake data.
- It is not clear whether intake data from dietary surveys in the general population accurately reflect consumption patterns in the allergic consumer, which could result in over or underestimates of the risk.
- Application of the methodology requires an understanding of the statistical background (e.g. choice of sampling methodologies, etc).

4. Discussion

The outcome of the workshop discussion is described below, grouped under the main topics raised.

4.1. Characteristics and quality of clinical data

The foundation of a good risk assessment is the quality and suitability of the data used. Food allergy risk assessment benefits from

the fact that the data used originate from the species of interest, but the use of existing data often incurs limitations because it was generated for other purposes. Early publications consisted of case reports and case series. These provided good hazard identification, but little in the way of characterisation. Where double-blind placebo-controlled food challenge (DBPCFC) data were generated, these aimed at diagnosing the individual patient rather than establishing even an individual NOAEL, let alone population ones. Other limitations of such studies with regard to their use for risk assessment include diverse protocols and quality of reporting, test materials, food matrices and patient characterisation.

In the last 5 to 10 years, DBPCFC procedures have been adapted to generate threshold data, as the value of such data both for clinical management of food allergy, and subsequently for public health purposes has become increasingly apparent. Several clinical trials specifically designed to determine NOAELs and minimum eliciting doses (also called LOAELs) for various allergenic foods have incorporated an extended range of doses, often starting at low micrograms levels. The most recent ones have also adhered fairly closely to a consensus clinical protocol (Taylor et al., 2004), facilitating data analyses within and across studies.

Studies designed to generate data for assessment of the risk to public health thus differ in important ways from those aimed simply at diagnosing individual patients. These different requirements must be reflected in study design and reporting.

4.2. Study design

A critical issue in the public health context remains the validity of extrapolating findings from the challenged population to the general allergic population. Exclusion from studies of individuals who have suffered a severe reaction is well documented and remains a concern to risk managers. However, the likelihood that the challenged population is biased towards more sensitive individuals who are difficult to treat is less acknowledged, yet has considerable implications for assessing risk. In particular, the predictive power of statistical models lies in a good understanding of the relationship between the sample used to build the model and the population it purports to represent. Thus, selection of participants in a trial is critical. In attempts to model population dose distributions, an ideal sample would therefore include the full range of subjects allergic to a specific food in the right proportions, including both low-dose and more typical reactors, i.e. a random sample of the allergic population. Alternatively a weighted sample could be used to improve the description of the population in areas where there are fewer individuals (e.g. low and high dose zones), but would still need to be accurately mapped onto the overall allergic population.

Dose escalation protocols should start at doses low enough to avoid first dose reactors, so that NOAELs can be generated, if possible. NOAEL data are to be preferred to LOAEL data for setting thresholds in the NOAEL uncertainty factor approach, but both parameters can be incorporated into statistical analyses. Dose number and spacing is also important to dose-distribution modelling approaches such as BMD, with greater number of doses and fewer reactors at each dose being preferable to relatively few doses and with larger numbers of reactors at each dose (Slob et al., 2005), though increasing the number of doses can make the challenge process more difficult and inconvenient for the participants.

4.3. Study reporting

Interpretation of food challenge studies for risk assessment requires as comprehensive a description of both the participants and the observations as possible in order to provide an understanding of the variability and uncertainty associated with any conclusions

drawn from them. Clinics do not generally have accurate information on the amount of food that triggered severe symptoms in their patients leading to exclusion in challenge trials. Therefore, it is not clear whether excluding such subjects will exclude subjects with an inherently greater sensitivity to the allergens or whether such subjects might be people with poor dietary management who therefore were more likely to have reactions. The rationale for including or excluding certain subjects (medication, severity) should be stated in published studies.

Studies comparing dose distribution in severe and moderately severe reactions are therefore highly recommended.

The traditional discrimination between subjective and objective symptoms needs reexamination. Severe GI-tract pain (subjective) may be a more important symptom than the occurrence of some hives (objective). Both types of symptoms should be reported. It may be helpful to also include some scoring of the severity of the specific symptom (such as mild/moderate/severe), since there is a large variability in the severity of symptoms, e.g. mild nausea, lasting only for 20 min and severe lasting nausea, which just did not lead to vomiting. Scoring systems have been proposed that take simultaneous account of both eliciting dose and severity of reaction elicited (Hourihane et al., 2005).

Both discrete and cumulative LOAELs should be reported. It is not possible to determine, and remains to be investigated, whether it is the discrete or the cumulative dose that causes the reaction. Data must be analysed separately for children and adults.

Workshop participants recognised that the amount of data generated in a challenge study, which would be of use to risk assessors, might be excessive for publication. However, detailed raw data should be made publicly available if they are not a part of the publication. Some journals have repositories for additional data which are accessible electronically.

4.4. Uncertainty factors

Depending on the goal of the risk or safety assessment, uncertainty factors may need to be applied. There is not yet a consensus on whether a single factor would suffice, or whether uncertainty factors could vary in different situations (e.g. different population groups). Participants discussed whether uncertainty factors should be set on a scientific or pragmatic basis and concluded that data are not yet available to be able to scientifically establish appropriate uncertainty factors. A factor of at least 10 is necessary but it is important to remember that the use of LOAELs and NOAELs may be considered as inherently conservative because they are derived from the more sensitive subjects in the population. Furthermore, there is no need for an uncertainty factor to account for inter-species differences, since all dose response data for allergenic foods are collected directly from studies in humans. However, other potential modulators of the allergic response such as severe, uncontrolled asthma, exercise, pollen exposure, intake of drugs like NSAIDs, or alcohol may need to be taken into account when appropriate uncertainty factors are set. Data from studies of oral desensitisation in food allergy that are likely to be carried out in the next few years may provide additional information in this area. Defining uncertainty factors will need to be informed by the objectives of food allergen management and the dynamics of allergic consumers' approach to risk. Using excessively large uncertainty factors with the laudable aim of preventing reactions in the most sensitive section of the allergic population may in practise result in a lower level of protection of this population. This could arise if manufacturers find themselves unable to assure that their products contain allergen below the mandated levels and resort to an extension of precautionary labelling. This could devalue the impact of such labelling and thereby increase, rather than reduce the risk.

4.5. Mathematical modelling of challenge data

Modelling makes use of all the relevant data, including the slope of the dose-distribution curve, in contrast to approaches based on a NOAEL. Data from different studies should, where possible, be combined to increase the statistical power of the analysis. However, before data can be combined, they should be subjected to individual analyses to determine how homogeneous they are. Variables that should form part of this preliminary analysis include age distribution, endpoints (subjective or objective responses), and selection of the study population.

Workshop participants agreed that the MoE should be calculated using the Benchmark Dose lower limit of the 95%-confidence interval as the point of departure. Compared to the use of a NOAEL as a point of departure, this ensures that the whole dataset contributes (as discussed above). Furthermore, the lower limit of the 95%-confidence interval takes account of the variation around the BMD in a conservative manner. Initially, the BMDL₁₀ can be applied, given the limited amount of data available for most allergenic foods. However, the BMDL₅ or BMDL₁ could be used as data became available.

Crump (1984) originally proposed the BMDL₁₀ as the point of departure because he observed a background incidence of that order in controls of many toxicological findings. While the incidence of objective reactions to the placebo is normally 0% in challenge studies, there is often a measurable incidence of subjective reactions to placebo. This background response to placebo could logically constitute the Benchmark Dose when subjective responses are analysed.

As noted earlier, the quality of the output of modelling approaches depends on good study design. Dose spacing and the number of doses are critically important parameters in producing good descriptions of the population distribution of MEDs (Slob et al., 2005). Clearly, good representation of the population also depends on correct sampling. Validation of predictions from dose-distribution modelling could be achieved by challenging a large number of randomly chosen allergic individuals with a single dose equivalent to the amount predicted to be safe in 90% of allergic individuals (this relatively low value is designed to assure the safety of such a procedure even for highly reactive individuals).

4.6. Exposure data

A reaction to a food allergen is analogous to an episode of acute poisoning rather than chronic toxicity in terms of dosimetry. Therefore the relevant exposure assessments should be based on 'meal/eating occasions' (to include snacks and impulse products) rather than exposure throughout the entire day or from a single food.

There is a need to obtain data on the behaviour of food allergic consumers, including patterns of food choices and whether this is the same in the most and least risk-averse food allergic consumers because this will affect any risk calculation. For instance, if most allergic consumers avoid a particular type of product, the risk posed by this product (in terms of the probability that it will cause a reaction if it contains a defined amount of allergen) will be lower. The extent to which this occurs need to be understood when exposure data are used to validate model predictions.

4.7. Generating data on all major food allergens

It may take many years to generate relevant data for all the major food allergens. Before all data are available, a pragmatic approach, as an interim measure, would be to read across from those allergens where data are available and then subsequently re-

visit the acceptable management thresholds as data on the various allergens become available.

4.8. Risk assessment/safety assessment

The NOAEL/uncertainty factor and the MoE approach can be used for safety assessment, i.e. is this product safe or not? These methods cannot be used to assess risk, i.e. what is the probability that an adverse incident will occur?

The workshop concluded that data from LOAELs and NOAELs, together with uncertainty factors, can be used in regulatory decisions, e.g. whether certain derived products should be exempt from allergen labelling. These data are less useful for derivation of allergen management thresholds for use by industry in routine food production. As discussed earlier, this could result in excessive precautionary labelling and ultimately in an increase rather than a decrease in the risk to allergic consumers. The inability of the current analytical methodology to detect such very low levels would also make them of questionable value in determining compliance.

MoEs are indicative numbers that can be used to prioritise areas of concern for risk managers. For example, very large/wide MoEs indicate that efforts do not need to be spent further improving cleaning procedures for some allergens. MoEs are thus complementary to the risk assessment process.

Probabilistic modelling is useful for quantitative risk assessment and in risk analysis. It uses quantitative data and can calculate the probability of a reaction occurring within a population, given a particular dose distribution of allergen as a starting point. Alternatively, it can be used to calculate maximum allergen concentrations using a maximum accepted risk as a starting point. Of course, risk assessment is an iterative process which is repeated until risk reduction measures lead to an acceptable level of risk. The process itself provides information about which measures are most effective and helps to prioritise risk reduction measures.

For all approaches (NOAEL/uncertainty factor approach, MoE approach, and probabilistic quantitative risk assessment approach), availability of reliable data will determine the possibilities and limitation of the application of the approach.

5. Conclusions

The workshop concluded that all three approaches to safety and risk assessment of allergenic food should continue to be considered. A particular strength of the MoE and Probabilistic approaches is that they do not rely on low-dose extrapolation. However, probabilistic modelling is considered to be the most promising approach for use in population risk assessment (which is a particular focus for risk managers in regulatory agencies and industry). As for the other approaches, further improvement of input data is desired, particularly data on consumption patterns/food choices in food allergic consumers, MED data for additional allergenic foods and data to evaluate whether the whole population at risk has been modelled accurately. However, as long as the optimal input data are still under development, the method can be applied using worst case or conservative assumptions in the risk assessments and read across approaches. The use of NOAELs and LOAELs was considered to be more appropriate for regulatory purposes, such as the evaluation of highly processed products derived from allergenic ingredients for exemption from statutory labelling requirements.

The use of these models in risk assessment is an iterative procedure, with further refinement of the calculations as more detailed and complete data become available.

With all approaches, it was noted that particular management thresholds that may be derived are for use at the population level

and cannot be used by an individual food allergic person in the management of their allergy.

The workshop identified a number of areas where risk managers need information on how to set allergen management levels in foods.

Current legislation on labelling of allergenic food is based on hazard identification. The workshop agreed that, in theory, legislative requirements for the labelling of allergenic ingredients should take account of allergen thresholds and could be informed by the establishment of NOAELs and LOAELs and by using agreed uncertainty factors. Such an approach could be used in future considerations of highly processed ingredients derived from allergenic foods as well as very low doses of the allergenic food itself.

The use of precautionary ('May Contain') labelling is not covered directly by current legislation in the European Union and most legislatures. The consequence is that the carryover levels that trigger the use of 'May Contain' labels vary between companies making the consequence for the consumer obscure. The workshop agreed that in theory thresholds for precautionary labelling, such as "May Contain", were possible but that there needed to be considerable discussion on the degree of risk that was acceptable at the population level. This discussion needed input from consumer groups, industry and regulators, as well as from clinicians, immunologists and toxicologists. The discussions should also include a consideration of whether there was a level of unavoidable cross contamination above which labelling the allergen as an ingredient (rather than as 'May Contain') could be appropriate. It was noted that current legislation in some regions might prevent the inclusion in an ingredients' list of an allergen that was not deliberately used in the product, although inclusion in voluntary labelling advice (such as 'Contains' boxes) might be possible. It was noted that whilst this approach might be applicable for homogeneous cross contamination (e.g. where the allergenic component was a liquid or powder), it would be a different situation for cross contamination that was likely to be discrete and sporadic (e.g. pieces of nut), which could be assumed to be a hazard for all people allergic to that food.

A prerequisite for moving from risk assessment to risk management includes an agreement on tolerable risk. While agreement still needs to be reached with all stakeholders on the proportion of the population for which general manufactured foods can reasonably be guaranteed to be safe and on the types of reaction they should be protected from, existing legislation or regulatory opinion may offer some pointers. Thus the US's Food Allergen Labelling and Consumer Protection Act talks of reactions that "pose a risk to human health" while EFSA Opinions on substances for which exemptions from allergen labelling have been granted have all referred to the view that they "were not likely/not very likely/unlikely to trigger a severe allergic reaction".

Whilst, on a scientific and theoretical basis, varying the regulatory or management thresholds for different allergens might be desirable, account needs to be taken of the practicalities of the way the management thresholds would be used in industry. It was suggested that risk assessment models could generate management threshold information on terms of amounts of the allergenic food consumed in a single eating occasion and that industry could then use those amounts to determine appropriate concentrations of the allergenic food in the particular products that they were manufacturing and control strategies to achieve them.

Analytical methods and their performance were not discussed extensively at the workshop as they are the means whereby data are generated which can be used in risk assessment, rather than an element of the risk assessment. However, robust and accurate analytical methods, capable of measuring allergens in a range of food matrices, will become increasingly important as other data available for doing risk assessment (e.g. threshold data) also in-

crease in quantity and quality. Hence development and validation of analytical methodologies, together with reference materials also continues to be a high priority.

6. Research needs

To move the area of food allergy risk assessment forward, the workshop agreed that research is needed to answer the following questions.

6.1. Epidemiology

- What is the prevalence of food allergy?

6.2. Clinical

- Can potential confounders for sensitivity or severity of the allergic reaction such as severe, uncontrolled asthma or exercise be quantified?
- Do MEDs change with physiological state, for example with age?
- Can the scoring system used in challenge studies be improved or validated to better reflect severity rather than just subjective versus objective symptoms?
- How does the dose response distribution for severe (including anaphylactic) allergic reactions compare with the dose response distribution for moderately severe or mild reactions?

6.3. Risk assessment

- What criteria can be used to determine uncertainty factors to be applied to NOAELs and LOAELs?
- Can data from challenge studies be used to set NOAELs and LOAELs that represent the allergic population or will there be very sensitive persons with severe symptoms that are never challenged, resulting in data not representative for the whole allergic population?
- Can data from challenge studies be used to describe the distribution of sensitivities in the allergic population to establish Benchmark Doses or be used in probabilistic risk assessment? If there are very sensitive persons with severe symptoms that are never challenged what will be the consequence for the distribution and hence for the risk assessment?
- Is the mathematical modelling of challenge and exposure data appropriate?
- What are the data requirements for modelling, e.g. number of patients and the criteria for combining studies?
- Which criteria should be used when using read across data on management thresholds for one allergen to another?

6.4. Other

- Can dietary surveys from the general population be used in exposure estimates in food allergy?
- Are food consumption patterns and choice of food products different in food allergic compared to non-allergic persons and do they differ in the most and least risk-averse subjects?
- Is it possible prospectively (e.g. through national registries) to follow food allergic reactions occurring in the community and get valid data on foods most likely to cause reactions as well as eliciting dose versus severity data from real life situations?

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors want to thank the workshop participants and especially Rachel Ward, André Knulst, Elisabeth Vavasour and Josef Schlatter for valuable comments to the manuscript.

The workshop was funded by the European Commission contract no. 514000 EuroPrevall “*The Prevalence, Cost and Basis of Food Allergy across Europe*” and by the UK Food Standards Agency, and organised in collaboration with the ILSI-Europe Food Allergy Task Force.

References

- Bock, S.A., Lee, W.-Y., Remigio, L.K., May, C.D., 1978. Studies of hypersensitivity reactions to foods in infants and children. *Journal of Allergy and Clinical Immunology* 62, 327–334.
- Codex Alimentarius Commission, 2003. Draft Principles for the Risk Analysis of Foods Derived from Modern Biotechnology, at Step 8 of the Elaboration Procedure. ALINORM 03/34, Appendix II. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organisation, Rome. Available from: <<http://ftp.fao.org/codex/alinorm01/al0134ae.pdf>>.
- Crevel, R.W.R., Briggs, D., Hefle, S.L., Knulst, A.C., Taylor, S.L., 2007. Hazard characterisation in food allergen risk assessment: the application of statistical approaches and the use of clinical data. *Food and Chemical Toxicology* 45, 691–701.
- Crump, K.S., Company Inc., 1984. A new method for determining allowable daily intakes. *Fundamental and Applied Toxicology* 4, 854–871.
- EFSA, 2004. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission relating to the evaluation of allergenic foods for labelling purposes. *The EFSA Journal* 32, 1–197.
- Fagt, S., Christensen, T., Biloft-Jensen, A., Matthiesen, J., Trolle, E. Meal Habits among Children and Adolescents 2000–2004. National Food Institute 2007 (in Danish with English summary).
- FAO/WHO, 1997. Risk Management and Food Safety, Report of a Joint FAO/WHO Consultation, Rome, Italy, 27 to 31 January 1997, FAO Food and Nutrition Paper 65. Food and Agriculture Organisation of the United Nations, Rome. Available from: <<http://www.fao.org/docrep/W4982E/W4982E00.htm>>.
- Flinterman, A.E., Pasmans, S.G., Hoekstra, M.O., Meijer, Y., Hoffen, E.V., Knol, E.F., Hefle, S.L., Bruijnzeel-Koomen, C.A., Knulst, A.C., 2006. Determination of no-observed-adverse-effect levels and eliciting doses in a representative group of peanut-sensitized children. *Journal of Allergy and Clinical Immunology* 117, 448–454.
- Food Standards Agency, 2006. Guidance on Allergen Management and Consumer Information – Best Practice Guidance on Managing Food Allergens with Particular Reference to Avoiding Cross-Contamination and Using Appropriate Advisory Labelling (e.g. ‘May Contain’ labelling). Published by the UK Food Standards Agency. Crown Copyright. Available from: <<http://www.food.gov.uk/multimedia/pdfs/maycontaininguide.pdf>>.
- Food Standards Agency, 2002. Nut Allergy Labelling, Report of research into the Consumer Response. Food Standards Agency, UK.
- Hefle, S.L., Furlong, T.J., Niemann, L., Lemone-Mule, H., Sicherer, S., Taylor, S.L., 2007. Consumer attitudes and risks associated with packaged foods having advisory labeling regarding the presence of peanuts. *Journal of Allergy and Clinical Immunology* 120, 171–176.
- Hourihane, J.O’B., Kilburn, S.A., Nordlee, J.A., Hefle, S.L., Taylor, S.L., Warner, J.O., 1997. An evaluation of the sensitivity of peanut allergic subjects to very low doses of peanut protein: a randomised, double-blind, placebo-controlled food challenge study. *Journal of Allergy and Clinical Immunology* 100, 596–600.
- Hourihane, J.O’B., Grimshaw, K.E.C., Lewis, S.A., Briggs, R.A., Trewin, J.B., King, R.M., Kilburn, S.A., Warner, J.O., 2005. Does severity of low-dose, double-blind, placebo-controlled food challenges reflect severity of allergic reactions to peanut in the community? *Clinical and Experimental Allergy* 35, 1227–1233.
- Kroes, R., Galli, C., Munro, I., Schilter, B., Tran, L.-A., Walker, R., Würtzen, G., 2000. Threshold of toxicological concern for chemical substances present in the diet: a practical tool for assessing the need for toxicity testing. *Food and Chemical Toxicology* 38, 255–312.
- Kruizinga, A.G., Briggs, D., Crevel, R.W.R., Knulst, A.C., van den Borch, L.M.C., Houben, G.F., 2008. Probabilistic risk assessment model for allergens in food: sensitivity analysis of the minimum eliciting dose and food consumption. *Food and Chemical Toxicology* 46, 1437–1443.
- Lam, H.Y., van Hoffen, E., Michelsen, A., Guikers, K., van der Tas, C.H., Bruijnzeel-Koomen, C.A., Knulst, A.C., 2008. Cow’s milk allergy in adults is rare but severe: both casein and whey proteins are involved. *Clinical and Experimental Allergy* 38 (6), 995–1002 (Epub 2008 Apr 1).
- Larsen, J.C., 2006. Risk assessment of chemicals in European traditional foods. *Trends in Food Science and Technology* 17, 471–481.
- May, C.D., 1976. Objective clinical and laboratory studies of immediate hypersensitivity reactions to foods in asthmatic children. *Journal of Allergy and Clinical Immunology* 58, 500–515.
- Slob, W., Moerbeek, M., Rauniomaa, E., Piersma, A.H., 2005. A statistical evaluation of toxicity study designs for the estimation of the Benchmark Dose in continuous endpoints. *Toxicological Sciences* 84, 167–185.
- Spanjersberg, M.Q.I., Kruizinga, A.G., Rennen, M.A.J., Houben, G.F., 2007. Risk assessment and food allergy: the probabilistic model applied to allergens. *Food and Chemical Toxicology* 45, 49–54.
- Taylor, S.L., Hefle, S.L., Bindslev-Jensen, C., Atkins, F.M., Andres, C., Bruijnzeel-Koomen, C., Bursk, A.W., Bush, R.K., Ebisawa, M., et al., 2004. A consensus protocol for the determination of the threshold doses for allergenic foods: how much is too much? *Clinical and Experimental Allergy* 34, 689–695.
- Threshold Working Group, 2008. Approaches to establish thresholds for major food allergens and for gluten in food. *Journal of Food Protection* 71, 1043–1088.
- Wensing, M., Penninks, A.H., Hefle, S.L., Koppelman, S.J., Bruijnzeel-Koomen, C.A.F.M., Knulst, A.C., 2002. The distribution of individual threshold doses eliciting allergic reactions in a population with peanut allergy. *Journal of Allergy and Clinical Immunology* 110, 915–920.
- World Health Organization, 1987. Principles for the Safety Assessment of Food Additives and Contaminants in Food. *Environmental Health Criteria* 70. International Programme on Chemical Safety, Geneva. Available from: <<http://www.inchem.org/documents/ehc/ehc/ehc70.htm>>.