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MEETING REPORT

RISK ASSESSMENT OF FOOD ALLERGENS

PART 2: REVIEW AND ESTABLISH THRESHOLD LEVELS IN FOODS FOR THE PRIORITY ALLERGENS

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
WORLD HEALTH ORGANIZATION
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ABBREVIATIONS AND ACRONYMS

BMD(L)	Benchmark dose (lower bound of 95% confidence interval)
BMR	benchmark response
CAC	Codex Alimentarius Commission
CCFH	Codex Committee on Food Hygiene
CCFL	Codex Committee on Food Labelling
CD	coeliac disease
CI	confidence interval
CM	cow's milk
DBPCFC	double-blind, placebo-controlled food challenge
EDp	the eliciting dose predicted to provoke reactions in a specified percentage (p) of the allergic population
ED_{01}	the eliciting dose predicted to provoke reactions in 1% of the allergic population
ED_{05}	the eliciting dose predicted to provoke reactions in 5% of the allergic population
ED_{10}	the eliciting dose predicted to provoke reactions in 10% of the allergic population
ED_{50}	the eliciting dose predicted to provoke reactions in 50% of the allergic population
EHC	Environmental Health Criteria
ELISA	enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
FBO	food business operators
FC	food challenge
FEV	forced expiratory volume
GPFH	General Principles of Food Hygiene
GSLPF	General Standard for the Labelling of Prepackaged Foods
HBGV	health-based guidance value
IgE	immunoglobulin E
JECFA	Joint FAO/WHO Expert Committee on Food Additives

Left censored	When a subject shows challenge-stopping objective symptoms at the first dose (i.e. no NOAEL can be established), the first dose is considered the LOAEL, and the data are considered to be left censored because the subject's true threshold is at or less than the first dose (Westerhout *et al.*, 2019).
LOAEL	lowest observed adverse effect level
MED	minimum eliciting dose
MoE	margin of exposure
MS	mass spectrometry
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
OFC	oral food challenge
PAL	precautionary (advisory) allergen labelling
RfD	reference dose
Right censored	When a challenge is completed or stopped without observation of objective symptoms (i.e. no objective LOAEL can be established) but the subject is considered to be allergic based on the clinician's judgement, the final discrete or cumulative dose given is then designated the NOAEL. In such a case, the data are considered to be right censored because the dose that would elicit objective symptoms is assumed to be greater than the last given dose (Westerhout *et al.*, 2019).
SF	safety factor
SMA	stacked model averaging
UAP	unintended allergen presence
UF	uncertainty factor
WDEIA	wheat-dependent exercise-induced anaphylaxis
WHO	World Health Organization

DECLARATIONS OF INTERESTS

All participants completed a Declaration of Interests form in advance of the meeting. Three of the experts declared interest in the topic under consideration. Markus Lacorn and Eva Södergren declared significant interests connected with their employment, and Clare Mills declared interests connected to investments that exceeded the FAO/WHO's threshold. It could not be excluded that the declared interests may be perceived as a potential conflict of interest. Therefore, while all three persons mentioned above had been invited to participate in the meeting, they were excluded from the decision-making process regarding final recommendations and participated as technical resource people.

All remaining experts were not considered by FAO and WHO to have declared any interest that may be perceived as a potential conflict with regard to the objectives of the meeting.

All the declarations, together with any updates, were made known and available to all the participants at the beginning of the meeting.

All the experts participated in their individual capacities and not as representatives of their countries, governments or organizations.

EXECUTIVE SUMMARY

Knowledge of thresholds constitutes a critical requirement to assessing the risk from allergens, as they are a characteristic of the hazard that allergens present to the food-allergic population. Their establishment, which is a focus of the terms of reference (ToR) for the second meeting, is thus essential to evidence-based application of risk management and mitigation strategies, such as precautionary allergen labelling (PAL).

The expert committee followed the ToR as formulated, except that they considered the list of priority allergens decided at the first meeting of this FAO/WHO Consultation. The ToR clearly signalled that the thresholds that the Codex Committee on Food Labelling (CCFL) looked to being defined were health-based guidance values (HBGV). Guided by the definition of HBGV in Environmental Health Criteria 240 (EHC) Chapter 5, the expert committee considered and deliberated four approaches to define thresholds (analytical-based, no observed adverse effect level [NOAEL] + uncertainty factor [UF], benchmark dose [BMD] with/without margin of exposure, and probabilistic hazard assessment) with the focus being on identifying which one(s) were most suited to derive an HBGV as defined above. After discussion of each approach, the expert committee concurred that the benchmark dose/probabilistic hazard assessment approach aligned most closely with the requests of the Codex Committees.

The approach having been defined, the expert committee was then able to discuss and agree on the safety objective. The expert committee agreed that it could be described as:

> to minimise, to a point where further refinement does not meaningfully reduce health impact, the probability of any clinically relevant objective allergic response, as defined by dose-distribution modelling of minimum eliciting doses (MEDs) and supported by data regarding severity of symptoms in the likely range of envisioned Reference Doses (RfD).

The expert committee further identified several important considerations to guide decisions. These included a clear definition of criteria to be met by quantitative data on which reference doses (RfD) are based; supporting data on health manifestations (severity) at the proposed RfD; quality, quantity, availability and accessibility of data (for priority allergens), as well as how to deal with priority allergens for which information supporting one or more of those considerations was lacking.

The expert committee then considered the form of the outputs, starting from the eliciting dose (EDp) values predicted to result in reactions (as defined earlier) in no more than 1 percent (ED_{01}) and 5 percent (ED_{05}) of the allergic population for the priority allergens, values which have already been shown to be protective in other analyses. They agreed as a general principle that the RfD values should be expressed

as milligrams of total protein from the allergenic source and contextualized, taking into account the wider and possible unintended consequences. Importantly, they concluded that a guiding principle should be whether selecting a more stringent (lower) value would materially improve the public health impact.

Data availability and quality being critical to the sound derivation of EDp values, the expert committee discussed potential data sources. They noted that the data reported by Remington *et al.* (2020) and Houben *et al.* (2020) formed the most comprehensive and best-described sources available, both in terms of content and curation, with supportive peer-reviewed publications. Dose-distribution analysis methodology was similarly well described. The expert committee reviewed the data sources for each priority allergen, taking into consideration both included publications and those which had been collated but excluded, and the extent and type of bias in the data.

Characterizing the hazard forms a critical component of risk assessment and considers both the numbers of people with the relevant allergy who will be affected by exposure to any given amount and the characteristics of any reaction that may occur.

The first element is covered by dose-distribution modelling, which has been extensively studied and is now well understood and developed. The second element is an evaluation of the likely health impact. A key factor that impacts the health of allergic individuals is reaction severity. Severity is a complex and multidimensional concept with an ill-defined relationship to dose; as such, severity data suitable for modelling are limited. Two principal sources of data were reviewed: 1) evidence of anaphylaxis reactions at defined doses, and 2) data on symptoms associated with reactions up to and including the ED_{01}, ED_{05} and ED_{10} from data used in the Remington *et al.* (2020) and Houben *et al.* (2020) publications. The latter indicated that all symptoms up to ED_{05} fell into a mild or moderate category, while analysis of clinical data from controlled challenges indicated that up to 5 percent of reactions at both ED_{01} and ED_{05} could be classed as anaphylaxis, although none were severe, based on the World Allergy Organisation (WAO) definition.

Furthermore, the expert committee noted the extreme rarity of fatal food anaphylaxis (less than 1 per 100 000 person-years) and observed that no fatal reactions had been reported following exposure at or below amounts considered for RfD, i.e. the ED_{01} and the ED_{05}. Considering both the proportion of individuals potentially affected and the severity characteristics of reactions at ED_{01} and ED_{05}, including the absence of reports of fatal or severe anaphylaxis, the expert committee agreed that, for all priority allergens, the safety objective would be met by using the ED_{05} (evaluated using the data from the Remington *et al.* [2020] and Houben *et al.* [2020] publications) as the foundation for defining RfDs. This decision was also informed by the current analytical limitations over the use of ED_{01} versus ED_{05} as RfDs. The expert committee further simplified its recommendations to make their application easier. This was done by rounding the ED_{05} values down to one significant figure (mainly for allergens with some data limitations). Those foods with close ED_{05} values were then grouped together and a single value derived for the RfD,

further rounding down the value, if necessary. The resulting RfDs expressed as milligrams (mg) of total protein from the allergenic source were: 1 mg: walnut (and pecan), cashew (and pistachio) and almond; 2 mg: peanut, sesame seed, cow's milk and egg; 3 mg: hazelnut; 5 mg: wheat, fish, and 200 mg: crustacea. The expert committee further incorporated into their recommendations action levels, calculated for intakes of food (containing potential unintended allergens) ranging from 10 grams (g) to 1 000 g in 10 g increments.

Examining assay capability in relation to the recommended RfDs, the expert committee observed that RfDs can be implemented and monitored to some degree with current analytical capabilities but acknowledged that significant limitations on method performance exist. They strongly recommended that expression of analytical results be standardized as milligram (mg) total protein of the allergenic food per kilogram (kg) of food product analyzed in order to facilitate interpretation of results by users of analytical services. To address deficiencies in analytical methodology, they recommended the development of method performance criteria, as well as more extensive provision of accessible reference materials for the priority allergens. The expert committee also identified the need for better understanding of assay performance in different food matrices and greater transparency over assay-specific reagents, such as antibodies used in enzyme-linked immunosorbent assays (ELISA), which are critical to assay performance. Other areas for improvement identified include defined procedures for obtaining samples for analysis and for curation of samples for third party analytical laboratories.

CHAPTER 1
INTRODUCTION

At its 45th session in May 2019, the CCFL requested the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) to provide scientific advice to validate, and if necessary, update the list of foods and ingredients in section 4.2.1.4 of the *General standard for the labelling of prepackaged foods* (GSLPF) (FAO and WHO, 2019). This request was addressed at the first meeting of the Ad hoc Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens by first establishing the criteria for assessing additions and exclusions to the priority food allergen list, then evaluating the available evidence for foods of concern. The establishment of "thresholds below which the majority of allergic consumers would not suffer an adverse reaction" (FAO and WHO, 2020c) for the priority allergens identified at the first meeting forms part of the Codex requests.

In response to the requests from the Codex Committee on Food Hygiene (CCFH) (FAO and WHO, 2018), the objectives of the Expert Consultation were:

- What are the threshold levels for the priority allergens below which the majority of allergic consumers would not suffer an adverse reaction?
- For the priority allergens, what are appropriate analytical methods for testing food and surfaces?
- What should be the minimum performance criteria for these different analytical methods?

Thus, FAO and WHO reconvened the Ad hoc Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens for a second meeting to provide scientific advice on this subject.

The term "threshold" is used in many contexts, including (but not limited to) individual, clinical, analytical, regulatory and so forth. The phrasing of the terms of reference indicates that any proposed thresholds should be based on health outcomes and protect consumers with food allergies. Characterization of the risk according to levels of exposure underlies the derivation of those thresholds, while the population we are aiming to protect and against what adverse effects should be clearly described. It also implies consideration of issues such as what degree of risk is tolerable to those affected.

It is now well accepted that thresholds exist for food allergens as has been clearly demonstrated clinically through the use of titrated dose oral food challenges (OFC). Furthermore, in contrast to most data obtained to support food safety, they are based on experimental data obtained from human beings belonging to the sensitive population. Several different approaches have been used for the definition of thresholds in a food allergen safety context, each with their merits and drawbacks (Threshold Working Group, 2008; EFSA, 2014; Madsen *et al.*, 2020). Choice of approach, therefore, constitutes a starting point for the Expert Consultation.

The different contexts in which thresholds are used also implies consideration of the purposes for which they will be used. The terms of reference for the whole Expert Consultation indicate that the focus is on their application in the management of precautionary allergen labelling (PAL), but this itself can have multiple dimensions in addition to consumer health, such as decisions on recall, trade rejection, as well as advice to people with food allergies and outcome measures for food immunotherapy studies.

PAL originated possibly as early as the 1980s in an attempt by the food industry to remedy the issues arising from the lack of data to characterize the risk posed by unexpected/unintended allergen presence (UAP) in food products. It is consumer-facing and aims to warn people with food allergies that a product poses a risk. Since its inception, PAL has been increasingly misunderstood in terms of its regulatory status and distrusted, particularly among the people it is meant to protect. Much of this can be attributed to its voluntary nature and the lack of official, generally recognized standards around its application. Voluntary industry standards, such as the Australia-New Zealand Allergen Bureau's VITAL™ Program, which was specifically initiated in response to these issues, are being developed. The CCFH has adopted a Code of Practice on Food Allergen Management for Food Business Operators (FBOs) (FAO and WHO, 2020a) to address practices in the supply chain and production process. However, full implementation of the Code requires further scientific support through the establishment of thresholds (reference doses) for priority allergens to inform management of UAP.

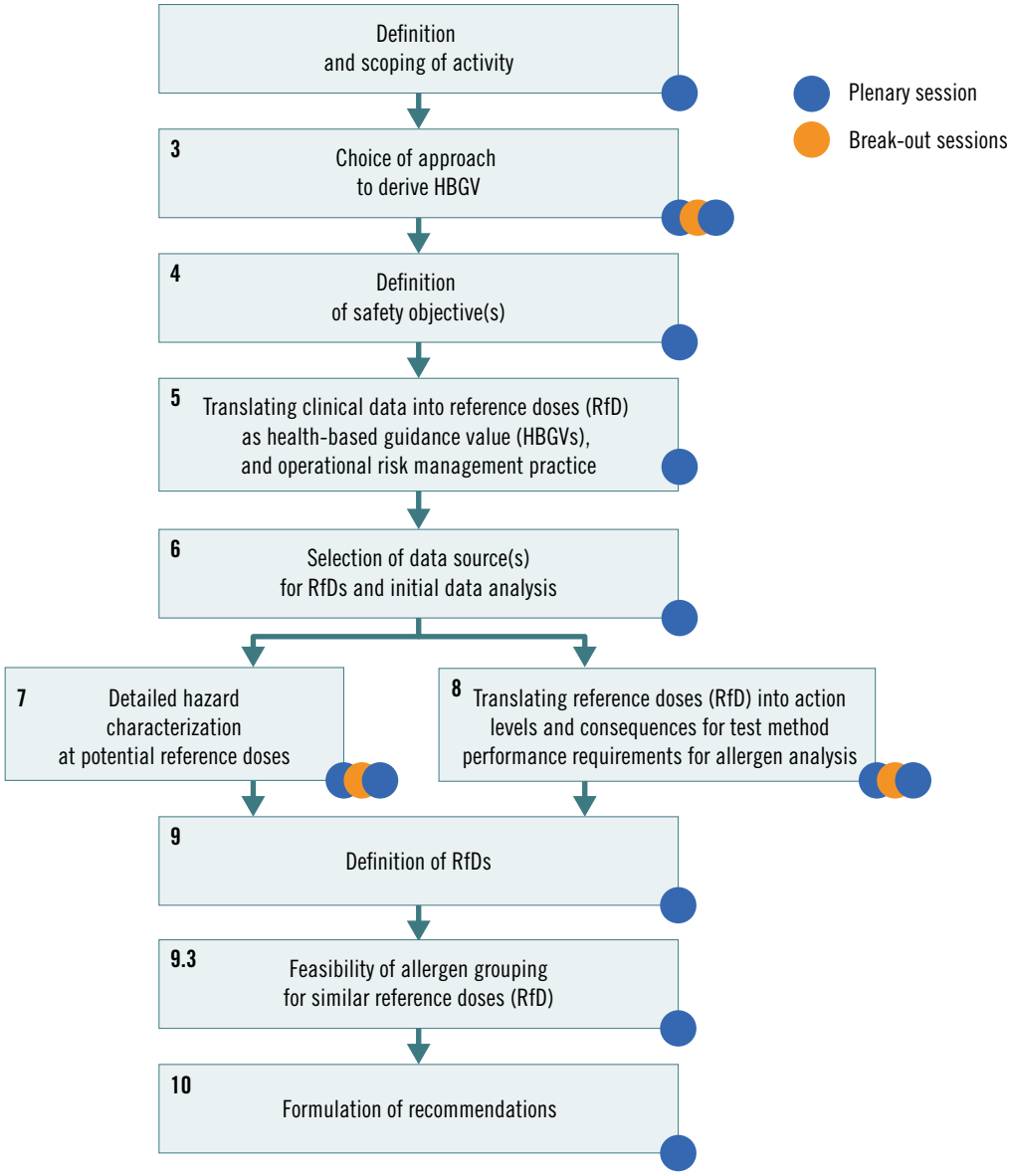
CHAPTER 2
OVERVIEW OF PROCESS

Figure 1 depicts the overall structure and flow of the process and logic adopted by the expert committee to derive its conclusions and recommendations.

Most discussions took place as plenary sessions, with consensus sought and achieved for the outputs. In the interests of efficient ways of working and to meet time constraints, the expert committee divided into break-out groups, which reported back to the whole expert committee with their conclusions and recommendations. Thus, for "Selection of approach to establish thresholds", four break-out groups were formed, each one to discuss one of the approaches. "Hazard characterization" and "Analytical capabilities" were each similarly discussed by one of two break-out groups. After conclusion of the break-out groups, any findings, results or outcomes were then summarized and discussed in plenary sessions to achieve consensus.

Numbers next to the boxes refer to the report sections.

FIGURE 1. **STRUCTURE AND FLOW OF THE PROCESS AND LOGIC ADOPTED BY THE EXPERT COMMITTEE TO DERIVE ITS CONCLUSIONS AND RECOMMENDATIONS**



Source: Authors' own elaboration.

Note: HBGV, health-based guidance value; RfD, reference dose; EDp, the eliciting dose predicted to provoke reactions in a specified percentage (p) of the allergic population.

CHAPTER 3
CHOICE OF APPROACH TO DERIVE HEALTH-BASED GUIDANCE VALUES (HBGV)

Several approaches have been and are used to derive health-based guidance values (HGBV) and other limits to support safety. These have been described and discussed in some detail (Threshold Working Group, 2008; EFSA, 2014; Madsen *et al.*, 2009). The expert committee divided into four sub-groups, each to consider one of four approaches and tasked with discussing for the relevant approach how well it could meet the objective of deriving an HBGV. For this purpose, HGBV were defined in Environmental Health Criteria 240 (EHC) Chapter 5 – 5.4.1 i.e. Health-based guidance values reflect a range of exposure without appreciable health risk (FAO and WHO, 2020b). The Committee's conclusions were then discussed in the plenary session.

The four approaches investigated and deliberated by the Expert Consultation were: analytical, deterministic safety assessment (no observed adverse effect level [NOAEL] with uncertainty factor [UF]), deterministic safety assessment (benchmark dose with/without margin of exposure [MoE]), and probabilistic hazard assessment. This built on the activities undertaken in the first working group where it was agreed that the potency measure for allergenic foods should be expressed as the dose of total protein from the allergenic source. Consequently, further description of doses of allergenic food used for derivation of HBGVs relate to the total protein content of an allergenic food or ingredient derived from such food.

Challenge data on various allergenic foods has become increasingly available over the past 20 years. More recently, many allergy clinics have been conducting baseline low-dose oral challenges as the initial phase of desensitization via immunotherapy. Several clinical challenge protocols were established for low-dose oral challenges (Taylor *et al.*, 2004; Crevel *et al.*, 2008; Varshney *et al.*, 2011; Cochrane *et al.*, 2012)

for acquisition of individual threshold data and subsequently for immunotherapy. Considerable variation occurs in the NOAELs and LOAELs between individuals with a given food allergy. For example, individual NOAELs for peanut within a large population from a single clinic ranged from 0.4 mg to 10 g of whole peanut, equivalent to 0.1 mg to 2.5 g of peanut protein (up to six orders of magnitude!) (Taylor *et al.*, 2010). When looking across clinics, it is now well-reported that individuals with food allergy can have LOAELs in clinical challenges that span up to eight orders of magnitude from 0.003 mg up to 8 000 – 10 000 mg of total protein from the allergenic source (Taylor *et al.*, 2010; Ballmer-Weber *et al.*, 2015; Remington *et al.*, 2020). At doses approaching an individual's LOAEL, objective symptoms are typically mild and resolve spontaneously when challenge doses are started at 1 mg or less of the offending food and the steps between doses are moderate (typically one-half log progression) as recommended in the various protocols.

3.1 ANALYTICAL-BASED APPROACH

The experts agreed that a process that is exclusively based on the capability of analytical procedures does not and cannot result in an HBGV. The logical sequence should be to first set an HBGV and then derive analytical values expressed as mg of total protein of the allergenic food per kg of food analysed. Regarding the methods to be considered for determining the concentration of allergenic protein, it is advisable to set method performance criteria (MPCs) instead of standardizing individual methods, since standardized methods may hamper the development of improved methods and technologies. It is worth noting here that in the absence of reference materials, the determination of accurate quantitative values for total protein from an allergenic food presents a challenge for the analyst. In a risk assessment context, the above-mentioned analytical values need to be considered in the context of the intake of the food of interest.

3.2 DETERMINISTIC SAFETY ASSESSMENT (NO OBSERVED ADVERSE LEVEL [NOAEL]/UNCERTAINTY FACTOR [UF])

The NOAEL/UF approach has been used in the field of toxicology for many decades. It is established by determining the no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL) (in case of absence of a lower NOAEL) based on all studies and all endpoints tested. To account for uncertainties and possible differences in experimental conditions with the actual human situation, the lowest NOAEL or LOAEL is usually divided by an uncertainty factor to derive an HBGV. The uncertainty factor (UF) used in toxicology varies between one and several hundreds or thousands. If there are no specific reasons to deviate, the usual default UF is 100. This factor is meant to account for possible interspecies (10-fold) and interindividual (10-fold) differences in toxicokinetics and toxicodynamics, i.e. to account for a possible higher sensitivity of humans compared to test animals and for the possible existence of a sensitive subpopulation among humans (Walton, Dorne and Renwick, 2001). The NOAEL/UF approach is essentially a "zero risk concept" approach.

When applied to the evaluation of food allergy threshold data, the default 10 × 10 UF does not apply, as the threshold data are derived from human observations and no animal-to-human extrapolation need be applied. The threshold data are also directly derived from challenges of the specific sensitive human subpopulation, i.e. the population who are allergic to that specific food. Thus, it is likely that the lowest NOAELs/LOAELs reported for this population would already include some of the most highly sensitive individuals within the population, particularly those generated by more recent studies.

For the purpose of recommending HBGVs for guiding the application of PAL, the NOAEL/UF approach, being a "zero risk concept" approach, would not be feasible or practical (Madsen *et al.*, 2020). It would result in extremely low HBGVs and consequently, extremely low action levels and required analytical quantification ranges. Such an approach would be highly unlikely to provide a practicable basis for meaningful, protective risk management and mitigation strategies, such as the application of PAL. The "zero risk concept" approach was not considered to be the desired model by multiple stakeholders in other meetings (see for example Threshold Working Group, 2008; Madsen *et al.*, 2009).

Due to these considerations, the subgroup did not recommend the NOAEL/UF approach for establishing HBGVs for allergenic foods.

3.3 DETERMINISTIC SAFETY ASSESSMENT (BENCHMARK DOSE WITH MARGIN OF EXPOSURE [BMD W/MoE])

The benchmark dose approach was originally described by Crump (1984) with the intention of making better use of dose-response results by deriving a point of departure using all the data, rather than a single NOAEL (or LOAEL) value.

A benchmark dose (BMD) is a dose or concentration that produces a predetermined change in the response rate of an adverse effect. This predetermined change in response is called the benchmark response (BMR). Normally, the default BMR is a 5 percent or 10 percent change in the response rate of an adverse effect relative to the response of a control group and is used to define a point of departure (either the BMD or BMDL – the lower bound of the BMD's 95 percent confidence interval) (Crump, 1984; EFSA, 2017). A desired margin of exposure (MoE) from the BMD(L) is established. If the actual MoE is less than the desired MoE, a health risk is assumed. The BMD(L) divided by the desired MoE is conceptually similar to the NOAEL/UF-based Health-Based Exposure Limit. Thus, it generally aims at a zero risk and consequently suffers from the same drawbacks, already discussed in section 3.2 above.

The BMD(L) can be used, and it provides useful information when it is not combined with an MoE, whereupon the BMD approach constitutes a probabilistic hazard assessment approach, characterizing the proportion of the relevant population at risk of experiencing the response as a function of dose. Therefore, the BMD approach fits the charge that "thresholds should be HBGV" as would a variation on this approach, as exemplified by the VITAL™ Program (Taylor *et al.*, 2014; Remington *et al.*, 2020).

Application of this approach would still need a debate about the accepted/tolerated level of risk (such as eliciting dose [EDp] value, and confidence intervals, etc). In addition, it would need to consider other factors, including study inclusion/exclusion criteria and harmonization and standardization of data expression from different studies to allow comparisons of outcome measures (such as symptom descriptions) and dose (e.g. conversion into total protein from the allergenic source). Clearly, it could also only be applied if the data available was of sufficient quality to develop dose-distributions.

If the MoE is not considered, then BMD and probabilistic hazard assessment approaches are equivalent. The subgroup therefore proposed that BMD w/MoE should not be taken further while BMD should.

3.4 PROBABILISTIC HAZARD ASSESSMENT

Probabilistic hazard assessment involves collection of the NOAEL and LOAEL data from an escalating low dose, oral challenges and modelling the dose-distributions using various parametric statistical models. These models allow prediction of the proportion of the population (p) of individuals allergic to protein from a specific food who will experience initial objective allergic reactions upon oral exposure to a dose (eliciting dose [ED]p) of total protein from that food (Taylor *et al.*, 2014; Remington *et al.*, 2020). The adverse reactions will be mild to moderate at each individual's minimum eliciting dose (MED), even with peanut which is widely considered an especially potent allergenic food (Hourihane *et al.*, 2017; Patel *et al.*, 2021a). The population ED-distribution provides a measure of the potency of an allergenic food. The foods with the lowest predicted ED values for a given proportion of the allergic population, e.g. the ED_{05}, would be the most potent. This probabilistic hazard assessment approach has been deemed the strongest, most transparent scientific approach for establish population thresholds (Buchanan *et al.*, 2008; Madsen *et al.*, 2009), but it is more demanding in terms of data inputs and their quality for deriving robust risk assessments, as discussed in the section on data requirements.

Parametric statistical models allow estimation of the ED_{01} and ED_{05} for a population even in circumstances where very few individual threshold data points are available. However, confidence in the estimates is enhanced when data from a sufficient number of patients are available. Statistically, estimates arising from groups of 60 or more patients are ideal, provided they are a representative sample (Klein Entink *et al.*, 2014).

Multiple parametric statistical models are available to examine the dose-distribution relationship among the individual threshold doses of groups of patients with specific food allergies. No biological reason exists to select one model over another (Taylor *et al.*, 2014). Thus, in early efforts, several estimates of population ED values were made using various models (Taylor *et al.*, 2010; 2014). Recently, stacked model averaging (SMA), employing multiple models, has been developed and used to obtain single population ED estimates based on input from commonly used parametric models and all dosing schemes/intervals (Remington *et al.*, 2020; Wheeler *et al.*,

2021). SMA is the preferred approach because it includes all models, uses predictive inference assigned weights to the various models based upon predictive accuracy, and best accounts for study-to-study heterogeneity (Wheeler *et al.*, 2021).

Probabilistic hazard assessment has several advantages over other approaches that were considered by the expert committee. This approach uses all the clinical data and allows comparison of differences between datasets from different clinics, studies or study types. Low-dose extrapolation is not needed. Uncertainty factors are not needed by definition. First-dose reactors and last-dose non-reactors can be included in the assessment using interval censoring survival analysis techniques (Taylor *et al.*, 2009). When dose-distribution data are combined with analytical data and food consumption information, risk managers can obtain predictions of the size of the population that may be at risk in a particular scenario.

The subgroup proposed carrying a probabilistic hazard assessment forward as an approach to consider as part of the establishment of population thresholds (reference doses, RfDs). The proposal was endorsed in plenary by the entire expert committee.

3.5 SUMMARY OF DISCUSSIONS AND CONCLUSIONS ON APPROACH TAKEN FORWARD

During a plenary meeting the subgroups presented the outcomes of their work as described in sections 3.1–3.4. Following plenary discussion of the different models, the expert committee agreed that the probabilistic hazard assessment/benchmark dose (without MoE), using dose-distribution modelling, should form the starting point and be the basis for the derivation of HBGVs for the priority allergenic foods already identified. The experts further decided that, rather than directly proposing a single population-based eliciting dose (EDp) value for each allergenic food, they would initially consider a range of options, identifying the implications of each option for risk, after which a final recommendation could be given.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CHAPTER 4
DEFINITION OF SAFETY OBJECTIVES

Meeting the terms of reference and establishing for priority allergens "thresholds below which the majority of allergic consumers would not suffer an adverse reaction" required consideration of – and agreement on – the safety objectives. The expert committee agreed that the thresholds, hereinafter referred to as reference doses (RfD) should be health based and conform with the definition of health-based guidance values (HBGV) as enunciated and elaborated in Environmental Health Criteria 240 (EHC), Chapter 5 Section 5.4.1 (FAO and WHO, 2020b). They should therefore "reflect a range of exposure without appreciable health risk" while being based on the sensitivity and reactivity of the whole relevant allergic population. The expert committee also agreed that the data available pertained only to the ability of the specified priority allergens to elicit reactions in individuals who had already acquired the relevant food allergy. The use of reference doses can therefore only address management and mitigation of the elicitation phase of allergic reactions (NOT the sensitization phase, i.e. development of an allergy).

In defining the safety objective based on HBGVs, the expert committee reviewed a range of possible options for defining the purpose of the HBGV and the outcomes it aimed to mitigate. These ranged from preventing or minimizing the occurrence of severe (life-threatening) anaphylactic reactions to preventing or minimizing any allergic reaction, subjective or objective. It also took into account, as far as possible, the criteria for defining tolerable risk formulated by Murphy and Gardoni (2008) and discussed by Madsen *et al.* (2020). Taking into consideration the complex and unresolved relationship between food allergen exposure (dose) and severity of allergic reactions (Dubois *et al.*, 2018), including anaphylaxis, as well as the uncertainties associated with subjective responses, the experts agreed that the safety objective addressed by RfD should be to:

> minimise the probability of any clinically relevant objective allergic response, (as defined by dose-distribution modelling of minimum eliciting doses [MEDs]) to a point where further refinement does not meaningfully reduce public health impact.

This should be supported by data demonstrating that incidental symptoms likely to be elicited in the range of envisioned RfDs are of an acceptable severity.

4.1 CONSIDERATIONS TO ENSURE OUTPUTS MEET SAFETY OBJECTIVE(S)

The expert committee further discussed important considerations pertaining to each priority allergen to ensure that proposed reference doses met the criteria for HBGV enunciated in EHC, Chapter 5. These are summarized below and, where necessary, further elaborated in individual sections.

4.1.1 CLEAR DEFINITION OF CRITERIA DEFINING QUANTITATIVE DATA ON WHICH REFERENCE DOSES (RFD) ARE BASED

In order for data from different studies to be combined for analysis, it is essential that the basis for distinguishing a reaction from its absence is clear. Experience with food challenge studies has demonstrated that this requires this distinction to be founded on clear, unambiguous criteria (Westerhout *et al.*, 2019). Objective symptoms fulfil this requirement; they include any sign that is externally observable, e.g. a rash, hives, whereas subjective symptoms cannot be confirmed by clinical observers, e.g. pruritis, throat tightness (in the absence of reduced forced expiratory volume [FEV]). Objective symptoms can continue from dose to dose or be transient. While objective symptoms are used to establish whether a reaction has occurred or not at a particular dose, subjective (non-observable) symptoms reported by the patient should nevertheless be recorded as part of the documentation of challenges. Westerhout *et al.* (2019) provide a non-exhaustive list of both subjective and objective symptoms. Controlled ontologies are also being developed to support curation and harmonization of symptom data to derive minimum eliciting doses in the ThRAll project (Mills *et al.*, 2019) based on clinical protocols used for oral food challenges (Grabenhenrich *et al.*, 2017). Such challenge protocols provide critical metadata for analysis especially since the stopping criteria are driven by analysis of symptoms and may vary between studies.

4.1.2 SUPPORTING DATA ON HEALTH MANIFESTATIONS (SEVERITY) AT PROPOSED REFERENCE DOSES (RFD)

Dose-distribution modelling defines the quantitative dimension of the allergenic hazard as the proportion of the at-risk population predicted to react. However, the nature and intensity of the signs and symptoms (severity) experienced at proposed RfD form another critical component of hazard characterization. They should therefore be evaluated, which means evaluation of the likely range and pattern of severity at the selected RfD. Assessment of severity is a critical component of hazard characterization, but the relationship of severity to exposure is complex and depends on many factors other than the amount of allergen (Dubois *et al.*, 2018). Data are currently insufficient and inadequate to describe it mathematically, but clinical and epidemiological observations can provide relevant supporting data in relation to the amounts of allergen protein involved. The expert committee agreed that considerations for reproducibility of thresholds (day-to-day variations in individual thresholds), cofactors, matrix effects, and data confounders should also be considered. Additionally, data on anaphylaxis in controlled challenge studies could be used

in this context, based on an understanding that anaphylactic reactions according to accepted definitions (Cardona *et al.*, 2020) span a wide spectrum of severity/risk to life and include mild/non-severe reactions.

For more information, please see "Section 7: Detailed hazard characterization at potential RfD" of the report.

4.1.3 QUALITY, QUANTITY, AVAILABILITY AND ACCESSIBILITY OF DATA (FOR PRIORITY ALLERGENS AS LISTED IN CHAPTER 8 OF THE FIRST MEETING REPORT [FAO AND WHO, 2022])

Sufficient good quality data are the foundation of robust assessments. Factors that need to be evaluated include but are not limited to the number of data points, inclusion and exclusion criteria, dose progression, challenge stopping criteria, demographics of source population and representativeness of overall population allergic to the allergen of interest as well as derivation of the data points (e.g. individual NOAELs and LOAELs) from the raw challenge data.

Approaches based on probabilistic hazard characterization are dependent for the soundness of their outputs upon having dose-distribution data from a population that is representative of the total population of individuals with allergies to a specific allergenic food. Datasets from individual clinics can fail to meet this criterion because patient selection bias purposely occurs in some situations such as with immunotherapy trials that select the most highly sensitive patients. Individual NOAELs and LOAELs should ideally be collected in a consistent fashion, using well-specified inclusion and exclusion criteria, as has been accomplished with one large global dataset (Westerhout *et al.*, 2019). Since several forms of the allergenic food are used as challenge materials clinically, the NOAELs and LOAELs should be normalized to total protein concentrations from the allergenic source (Taylor *et al.*, 2014). By using these approaches and comingling data from multiple clinics across the globe, a representative dataset can be acquired (Remington *et al.*, 2020).

4.1.3.1 Modelling considerations

Inclusion and exclusion of particular datasets and use of partial datasets needs to be signaled and its consequences considered in relation to the representativeness of values derived. Choice of model, as well as exclusion of alternatives, must also be documented.

4.1.3.2 Validation

In situations where it is possible, validation in unselected populations provides valuable support to the assumption that derived RfD are representative of the whole allergic population, or it indicates the possibility of bias. Single-dose challenges can directly test the EDp value for a single RfD and have been used with peanut, milk and hazelnut data but are only feasible with allergenic foods with a relatively high prevalence (Hourihane *et al.*, 2017; Turner *et al.*, 2021).

Other possibilities include comparing predicted modelling outputs (number of reactions) with observed numbers of reactions and allergen exposure determined from prospective surveys of reactions in defined allergic populations, combined with analytical measurement of allergen content in the implicated food(s) (e.g. Blom *et al.*, 2018).

4.1.4 HOW TO PROCEED FOR ALLERGENS WHERE THE ABOVE CONSIDERATIONS CANNOT BE COMPLETELY ADDRESSED AT PRESENT

Approaches under this heading could include reading across from allergens for which all the requirements can be met, for instance from tree nuts with an adequate dataset to taxonomically related ones for which little data exist.

CHAPTER 5

TRANSLATING CLINICAL DATA INTO REFERENCE DOSES (RfD) AS HEALTH-BASED GUIDANCE VALUE (HBGVs), AND OPERATIONAL RISK MANAGEMENT PRACTICE

Informed by the definition of the safety objectives and presentations on alternative approaches to defining thresholds, the expert committee discussed in detail the options for deriving RfDs from available data. The experts agreed to present initially a range of RfDs for each priority allergen, based on EDp values which had already been shown to be protective, namely ED_{01} and ED_{05}, using published values based on the selected data sources (Remington *et al.*, 2020 and Houben *et al.*, 2020), as discussed in more detail in Section 6. In order to keep the possibility open of offering risk managers a range of possible scenarios, a rationale was considered to present a range of RfD values. The expert committee also discussed the possibility of grouping allergenic foods according to their ED_{01}/ED_{05} values and developing group RfDs to facilitate application by risk managers. However, the experts ultimately decided that it would be preferable to start by deriving RfDs for individual priority allergenic foods, rather than grouping those foods and then deriving group RfDs. Again, this left open the option of grouping and simplifying at a later stage, if feasible and desirable, while maintaining a higher degree of transparency.

The expert committee divided into two subgroups, one examining hazard characterization, namely the health impact of selecting ED_{01} and/or ED_{05} as the basis for RfDs, while the other considered the implications of choices of specific EDp values for analytical capabilities. The two groups reported back in plenary sessions at frequent intervals to keep each other appraised of their thinking and conclusions. Individual RfDs for each allergen were then discussed and agreed in plenary taking into consideration the conclusions on hazard characterization.

The expert committee further discussed how their conclusions and recommendations should be formulated beyond the derived RfDs themselves. They agreed as a general principle that the RfD values should be contextualized, taking into account the wider consequences of basing them on a lower versus a higher EDp value. They iterated that the primary purpose of deriving the RfD was to improve the management of unintended allergen presence (UAP) in foods, which includes but is not limited to the use of PAL. Aware of possible unintended consequences, they concluded that a guiding principle should be whether selecting a more stringent value would materially improve the public health impact.

The expert committee also considered the practicalities of using RfDs, in particular how the recommendations and conclusions could be made as easy as possible for risk managers to apply. Since the (allergenic) protein constitutes the hazard in an allergenic food, they agreed RfDs should be expressed as milligrams of total protein of the priority allergenic food. Since people with a food allergy react to an amount (mass [mg]) of allergenic protein usually contained within a manufactured or catered food product, and not to the concentration as such, whether UAP in such a food product will provoke a reaction depends on:

> how much food is consumed; and

> the concentration of unintentionally present (allergenic) protein in that food product.

Management of allergens requires consideration of these two factors. To facilitate use of RfDs, the expert committee agreed that the recommendations should list, for each allergenic food, the action levels (concentrations of UAP above which action, e.g. the use of PAL, is required) corresponding with different intake ranges (portion sizes) of the food product containing the UAP. They also concluded that analytical test results used to determine UAP should be expressed as mg total protein of the (priority) allergenic food/kg of the analysed food product and that a summary of analytical capability against requirements, highlighting the most significant gaps, should be included in the recommendations.

CHAPTER 6
DATA SOURCE(S): AVAILABILITY AND REVIEW

The choice of data sources and extraction of data therefrom constitute a critical element in the development of the potential outputs. The decision to establish reference doses (RfD) for allergens as HGBVs means that they rest on data from escalating dose clinical challenges. The probabilistic hazard assessment approach, chosen as the foundation for the development of RfDs, is dependent upon high quality data which meet specified criteria, as already briefly discussed in 6.1.3 above and upon having dose-distribution data from a population that is representative of the total population of individuals with allergies to a specific allergenic food. This section describes in more detail the general considerations around data used for dose-distribution modelling and then reviews against those considerations the data on individual priority allergens from the sources selected for this report (Remington *et al.*, 2020, Houben *et al.*, 2020). Additional details on the principles of data selection criteria can be found in Report 1, section 4.2.1 (FAO and WHO, 2022).

6.1 GENERAL CONSIDERATIONS THAT CAN IMPACT THE MODELLING OF THE THRESHOLD DISTRIBUTION AND ESTIMATION OF THE ELICITING DOSE PREDICTED TO PROVOKE REACTIONS IN A SPECIFIED PERCENTAGE (P) OF THE ALLERGIC POPULATION (ED_P)

The purpose of reference doses derived through dose-distribution modelling is to provide benchmarks of clinical reactivity to a food which are representative of the overall population of individuals allergic to that allergenic food. This requires that, as far as possible, the data used for modelling cover the whole range of minimum eliciting doses observed for individuals in a given allergic population and (priority) allergenic food. The distributions should be based on the analysis of both discrete or cumulative NOAEL and LOAEL doses from individual double-blind, placebo-controlled food challenges (DBPCFCs) for each priority allergen. To obtain distributions as representative as possible, data from multiple clinics and publications are aggregated.

In previous approaches, the statistical distribution – using interval-censoring survival analysis – that was fitted to the data was one of three parametric survival models: the log-logistic, the log-normal, or the Weibull distribution (Taylor *et al.*, 2014; Ballmer-Weber *et al.*, 2015). How well each particular model fitted the data at the lower and upper ends of the distribution generally varied according to the model and allergenic food. In some cases, the log-normal and log-logistic models fit best, with the Weibull distribution being over-conservative, while at other times the Weibull model provided the basis for the output (Taylor *et al.*, 2014). Model fitting statistics were unhelpful in choosing the most appropriate one. The drawbacks of using single model predictions are well-known. Other fields of toxicology and exposure-response assessment, in situations where the underlying mechanisms do not favour any single model, have moved to model averaging techniques in order to improve the outputs of resulting analyses (Chapter 5 of Environmental Health Criteria 240 [FAO and WHO, 2020b]; EFSA, 2017; US EPA, 2018).

Until recently, a model averaging method for interval-censored data was not available (Wheeler *et al.*, 2021). Accordingly, a "Bayesian stacked model averaging" method for interval-censored data was developed. The model combines predictions from multiple statistical dose-distribution models into one output by assigning weights to each model based on goodness-of-fit and by averaging the results across the available models based on these weights (Wheeler *et al.*, 2021). Data from multiple studies are combined, and the model includes random effects so that the study-to-study variability is taken into account (different locations, different protocols, different clinicians, etc.). However, while the Bayesian stacked model averaging could be more efficient since it is an all-in-one statistical package, for each statistical approach, the quality and the amount of data remain the most important factors to consider as well as validation against clinical observations, using objective symptoms as the preferred metric to decide whether a reaction is positive or not, as discussed earlier.

The **dosing scheme** in a threshold study is another important factor because it determines the dosage range covered and the increments between each individual dose. Different protocols have been used: EuroPrevall suggested a low-dose clinical consensus protocol for MED determination minimizing the number of first dose reactors, representing maximum coverage of the dosing scale. The EuroPrevall dosing levels were 0.003, 0.03, 0.3, 3, 30, 100, 300, 1 000 and 3 000 mg total protein from the allergenic source (Crevel *et al.*, 2008; Ballmer-Weber *et al.*, 2015 Sup Table E3). Not all dosing schedules follow this model, aimed at determining LOAELs and NOAELs, depending on their particular applications, such as diagnosis or immunotherapy. In the United States of America and the European Union, the PRACTALL consensus recommended a general challenge schedule consisting of 3, 10, 30, 100, 300, 1 000 and 3 000 mg of food protein at intervals of at least 20 minutes (Sampson *et al.*, 2012), which reflects much current clinical practice.

The effect of censoring observations due to the dosing steps influences the accuracy of any resulting EDp estimation (Klein Entink *et al.*, 2014). It is now well documented that individuals with food allergy can have LOAELs in clinical

challenge trials that span five to eight orders of magnitude – 0.003 mg up to 8 000–10 000 mg of total protein from the allergenic source (Taylor *et al.*, 2010; Ballmer-Weber *et al.*, 2015; Remington *et al.*, 2020). A dosing scheme that terminates at a comparatively low-dose (e.g. 100 mg of total protein from the allergenic source) will result in more right censored[1] subjects (i.e. a greater number of allergic individuals will fail to develop objective symptoms at the maximum dose used in the scheme than they would in a dose progression that finishes by delivering 1–3 g of protein). In contrast, a dosing scheme that is initiated at a comparatively high dose (e.g. 100 or 500 mg of total protein from the allergenic source) will result in more left censored[2] participants (i.e. a greater number of individuals who react with objective symptoms at the first dose administered in the scheme). However, having more left/right censored data does not necessarily lead to significant effects on model estimates for a given dose range (Klein Entink *et al.*, 2014) as a number of different factors can influence model estimates. Dosing steps in the higher dosing range have been shown to be necessary for an accurate representation of the threshold distribution (Klein Entink *et al.*, 2014). Overall, Klein Entink *et al.* (2014) reported that the loss of the three lowest dosing levels (i.e. below 3 mg protein in the EuroPrevall dosing scheme) has considerably less impact on the accuracy of EDp estimation than the loss of the three higher dosing levels (above 100 mg in the EuroPrevall dosing scheme). Therefore, the availability of data points along the whole threshold distribution (and therefore the dosing schedule) is important because if only low or high doses are tested, model estimates will be biased. Other considerations beyond modelling, such as participant safety, may, however, counsel starting food challenges at doses below 0.5–1 mg.

Sample size (i.e. number of individual thresholds) directly affects the accuracy of estimation. Overall, there is a tendency towards overestimation of the lower EDp's with small sample sizes (n<30) because patients are more likely to be sampled close to the median than to the lower tail of the distribution (Klein Entink *et al.*, 2014). The accuracy of estimation improved the most with each step in sample size from n = 20 to n = 60. For larger sample sizes, the marginal gains in accuracy and reduced bias declined so that a sample size of n = 60 or larger is recommended for obtaining stable estimates of threshold distributions from a representative population drawn preferably from more than one clinical centre (Klein Entink *et al.*, 2014). However, as reported in the simulation results by Klein Entink *et al.* (2014), the larger the sample size, the higher the probability that the ED_{01} lies close to the true population value.

[1] Right censored: when a challenge is completed or stopped without observation of objective symptoms (i.e. no objective LOAEL can be established) but the subject is considered to be allergic based on the clinician's judgement, the final discrete or cumulative dose given is then designated the NOAEL. In such a case, the data are considered to be right censored because the dose that would elicit objective symptoms is assumed to be greater than the last given dose (Westerhout *et al.*, 2019).

[2] Left censored: when a subject shows challenge-stopping objective symptoms at the first dose (i.e. no NOAEL can be established), the first dose is considered the LOAEL, and the data are considered to be left censored because the subject's true threshold is at or less than the first dose (Westerhout *et al.*, 2019).

POPULATION

For dose-distribution modelling to yield the best estimates of the true population threshold, the EDp value and individual NOAEL and LOAEL data should be obtained from a truly representative cross-section of the entire population with a specific food allergy (Crevel *et al.*, 2008). Achieving this requires challenges be performed preferably in unselected study populations, such as longitudinal cohorts, but the prevalence of food allergy means that where this has been undertaken, the size of the challenged population may be small (Grabenhenrich *et al.*, 2017; 2020; Nicolaou *et al.*, 2010; Osborne *et al.*, 2011; Peters *et al.*, 2017). Studies dedicated to the investigation of thresholds or dedicated to diagnostic studies using data from a clinical diagnostic perspective are usually drawn from outpatient clinic populations and are less biased and probably more representative of the expected results for the majority of the food-allergic population than those drawn from multicentre randomized controlled trials (RCTs) of food allergy therapeutics. Such RCTs generally use oral food challenges as an efficacy outcome measure and have potential to provide large datasets for dose-distribution modelling. Indeed, several studies have published threshold data including many results obtained from the placebo arms of such trials. However, patient inclusion criteria for such RCTs usually deliberately bias the study population towards more sensitive subjects in order to demonstrate a therapeutic effect. Consequently, individuals reacting to cumulative doses greater than 144 mg or 444 mg protein tend to be excluded. For peanut, this roughly corresponds to the ED_{40} and ED_{65}, respectively, when looking at results derived from large datasets drawn from outpatient clinic and unselected study populations (Houben *et al.*, 2020; Taylor *et al.*, 2009). Exclusion of more than half the individuals with a specific food allergy in this way will inherently shift the modelled dose-distribution curve to the left. Results from such RCTs should not be ignored but considered with acknowledgement of these limitations.

The responses of populations from different countries to allergenic foods can be influenced by the exposure pattern and dietary habits, which vary with culture/ethnicity. Sometimes, individual datasets tend to originate from only one or two geographic areas, which can be a limiting factor. However, if it seems important to provide data from different parts of the world, the effects of possible patient selection biases, clinical protocol differences, and other factors are reduced by combining data from various countries and multiple clinics (Allen *et al.*, 2014).

6.2 DATA REVIEW – GENERAL CONSIDERATIONS

The database reported by Remington *et al.* (2020) and Houben *et al.* (2020) is the largest and most comprehensive source of data for dose-distribution modelling, including both published data and unpublished data from cooperating allergy clinics. It is also actively maintained through a systematic search of the published literature on food allergens relating to allergen thresholds. Data quality criteria for inclusion and exclusion have been published in a peer-reviewed publication (Westerhout *et al.*, 2019). While the database itself is not publicly available, its outputs have been published in peer-reviewed publications (Remington *et al.*, 2020; Houben *et al.*, 2020) as have the dose-distribution modelling approaches and their use to derive reference doses (Taylor *et al.*, 2014, Wheeler *et al.*, 2021).

As detailed in Remington *et al.* (2020) and Houben *et al.* (2020), the authors used the criteria from Westerhout *et al.* (2019) to systematically search and update their publication database with results identified in databases such as PubMed and Scopus with the general search terms: (allergy AND [food OR nutrition] AND [DBPCFC OR challenge OR provocation OR threshold OR eliciting]). Publications with potential potency data were also added from a list of all publications relevant to food allergy as identified during a custom screening of Current Contents™, other literature databases such as Medline, scanning content pages of specialty allergy journals, and cross-referencing bibliographies of publications. Publications up to 2011 were identified, detailed and included in the analysis of Taylor *et al.* (2014). The database was further updated with publications between 2011–August 2018, with over 2 516 titles and abstracts screened for further review; 570 peer-reviewed articles were kept for full PDF review, and 47 were identified as containing quantitative individual level data in a useable format, as detailed and included in the analysis of Remington *et al.* (2020) and Houben *et al.* (2020).

For the current review, the Expert Consultation reviewed the dose-distributions as detailed in Remington *et al.* (2020) and Houben *et al.* (2020), as well as 71 publications identified by the Potency Subgroup in Part 1 of this Expert Consultation to potentially contain general group-level potency data (but previously identified not to contain detailed individual level data – and not included in the Houben *et al.* [2020] dose-distributions) (See Annex 1). These studies were identified after applying similar search criteria, abstract screening of nearly 3 000 publications, and a PDF review of more than 450 publications identified for detailed review. Furthermore, the subgroup reviewed additional studies identified for potential potency review by members of the current working group.

As part of the first meeting of the present FAO/WHO Expert Consultation, papers which were acquired for possible inclusion up to December 2020 were reviewed by members of the potency subgroup. The expert committee accepted a proposal that the outputs in Remington *et al.* (2020) and Houben *et al.* (2020) form the basis of reference dose derivation, subject to updating the review of non-included papers to March 2021. The expert committee then discussed the review and agreed that none of those latter papers contained data which would materially alter the dose-distributions for the allergenic foods of interest. The expert committee also reviewed at an additional plenary meeting held in March 2022 new publications on sesame seed and cow's milk that improved the robustness of RfD estimates for those allergenic foods. Available information for individual foods is detailed below.

6.3 WHEAT (*TRITICUM AESTIVUM* AND OTHER *TRITICUM* SPECIES)

IgE-mediated wheat allergy can be severe and usually develops during early infancy but frequently resolves by adolescence (Keet *et al.*, 2009; Kotaniemi-Syrjanen *et al.*, 2010).

For some allergic individuals, allergic reactions are elicited only when a triggering cofactor such as physical activity (exercise) is added around ingestion of wheat products.

Usually, clinical symptoms are elicited by exercise one to four hours around the intake of wheat products. This condition sometimes results in anaphylactic reactions and is denoted as wheat-dependent exercise-induced anaphylaxis (WDEIA). Other cofactors in WDEIA are the intake of acetylsalicylic acid (aspirin), other non-steroidal anti-inflammatory drugs (NSAID), alcohol, or the patient's general condition. In young adults and adolescents, anaphylactic reactions to wheat are most often food-dependent exercise-induced anaphylaxis (Morita *et al.*, 2007). The amount of wheat protein required to induce WDEIA has been characterized through challenge protocols which report patients ingesting large amounts of wheat protein before exercise or other cofactor challenges. As such, the amount of allergenic protein implicated in WDEIA is expected to be much higher than in celiac disease for which 10 mg daily gluten intake is considered safe (Akobeng and Thomas, 2008; Scherf *et al.*, 2016; Catassi *et al.*, 2007) (Table 1).

TABLE 1 **SUMMARY OF WHEAT-DEPENDENT EXERCISE-INDUCED ANAPHYLAXIS STUDIES DETAILS**

CHALLENGE FOOD	AMOUNT OF WHEAT PROTEIN	INTERVENTION	REFERENCE
200–400 g of udon noodles,	5.2–10.4 g of wheat protein		Asaumi *et al.* (2016)
100 g wheat flour	~10 g wheat protein	Before exercise	Wagner *et al.* (2016)
60 g of udon (wheat noodles) or 120 g of bread	32 g of gluten was eaten	Before exercise	Sugiyama *et al.* (2019)
Baked gluten rolls in increasing dosage (8, 16, 24 and 32 g)		Before cofactor challenges	Christensen *et al.* (2018)
Bread baked with 10 to 80 g of pure gluten flour	10 or 20 g increments up to 80 g of gluten	Cofactors (acetylsalicylic acid and alcohol) given 30 minutes prior to challenge which was followed by exercise	Brockow *et al.* (2015)

Source: Authors'own elaboration.

For people with wheat allergy, exposure to gluten (gliadins and glutenins) from wheat can trigger allergic reactions with many of the major wheat allergens belonging to gluten proteins (Juhász *et al.*, 2018). Wheat-allergic patients may benefit from a gluten-free diet (Hischenhuber *et al.*, 2006; Pietzak, 2012). At this concentration (<20 ppm), 100 g of gluten-free product consumed would expose wheat allergic individuals to a maximum of 2 mg of gluten.

AVAILABLE/ACCESSIBLE STUDIES

As detailed in the supplementary information from Remington *et al.* (2020), there are nine studies available for wheat (eight from published literature and one unpublished clinical dataset) with a total of 99 individuals included in the analysis (2 left-censored, 9 right-censored); 12 identified as adults. and 87 identified as children. In addition to the data from Remington *et al.* (2020) and Houben *et al.* (2020), four studies were identified for consideration for wheat (see Annex 1-Studies considered from potency subgroup review).

QUALITY/QUANTITY

In Part 1 of this Ad hoc Joint FAO/WHO Expert Consultation, the potency data from Remington *et al.* (2020) and Houben *et al.* (2020) were concluded to be an **adequate/good quantity of data** available (n = 99) for dose-distribution modelling. A **high/adequate potential for biases** was identified for the available data for wheat (FAO and WHO, 2022).

A *high to adequate* potential for biases with the available data was attributed because the population study was mainly composed of children (85 percent) since this allergy generally decreases with age, and the study participants came from eight countries but only two regions (Europe and Asia). The dosing scheme reported approximately 10 percent (11/99) of the dataset left- or right-censored and the allocation of data points along the threshold distribution (upper-, medium- and lower-end of the distribution) was balanced.

Among the 99 data points, 93 were obtained from published literature and six from unpublished studies. Among these six children, the first dose tested was 1.75 mg with no left-censored data. Among the 93 clinical data points remaining, the lowest dose tested was 2.6 mg and one patient reacted to this dose with objective symptoms among a population of 21 patients with positive oral food challenge (OFC) (Ito *et al.*, 2008).

The ED_{01} has been established at 0.7 mg ($CI_{95\%}$: 0.3, 2.5) and the ED_{05} at 6.1 mg ($CI_{95\%}$: 2.6, 15.6) for wheat with the Bayesian stacked model averaging methodology (Remington *et al.*, 2020). There is no single-dose challenge study available to verify the ED_{01} and the ED_{05} for wheat in an unselected outpatient clinic wheat-allergic population.

6.4 FISH

In the Codex Alimentarius, fish "*...means any of the cold-blooded aquatic vertebrate animals commonly known as such. This includes Pisces, Elasmobranchs and Cyclostomes*" (FAO and WHO, 2003, p. 1).

Fish intake varies considerably between different regions, depending on local traditions and supplies. Fish consumption also appears to vary greatly between families and individuals. Some patients may outgrow their fish allergy as reported for 3.5 percent of fish-allergic patients in one American study (Sicherer, Muñoz-Furlong and Sampson, 2004). The prevalence of fish allergy is higher in adults than in school-age children (see the first meeting report of this Expert Consultation). Different species of fish are eaten in different parts of the world although the impact of these differences on fish allergy remains unclear.

Parvalbumin, a muscle protein, is considered the predominant allergen in fish (Van Do *et al.*, 2005) and is considered responsible for cross-reactivity among fish species for many fish-allergic individuals (van Do *et al.*, 2005; Dijkema *et al.*, 2022). A multi-challenge study in fish-allergic subjects showed that codfish was the predominant allergenic fish, with 70 percent of subjects showing

cross-reactive allergies to either salmon or mackerel as well (Sørensen *et al.*, 2017). However, some individuals were monosensitized to either cod or salmon. These data support the view that some fish-allergic individuals may tolerate fish from taxonomically distinct orders while reacting to selected species (Bernhisel-Broadbent, Scanlon and Sampson, 1992; Liang *et al.*, 2017). Parvalbumin levels appear to vary widely among fish species (Griesmeier *et al.*, 2010; Lee *et al.*, 2011, Liang *et al.*, 2017), and a few fish-allergic individuals have been identified who do not react to parvalbumin but instead react to other fish proteins (Ebo *et al.*, 2010; Kuehn *et al.*, 2014). The levels of parvalbumin are generally lower in oily fish, possibly explaining in part the clinical cross-reactivity observed by Sørensen *et al.* (2017). Furthermore, the low levels of parvalbumin in cartilaginous fish species explain why individuals allergic to boney fish species can tolerate fish such as ray (Kalic *et al.*, 2019).

AVAILABLE/ACCESSIBLE STUDIES

As detailed in the supplementary information from Remington *et al.* (2020), there are five studies available for fish (four from published literature and one unpublished clinical dataset) with a total of 82 individuals included in the analysis; 29 identified as adults, and 19 identified as children. Regarding clinical datasets, most data used for dose-distribution modelling are from cod (n = 64), followed by salmon (n = 7), catfish (n = 5) and mackerel (n = 2).

QUALITY/QUANTITY

In Part 1 of this Ad hoc Joint FAO/WHO Expert Consultation (FAO and WHO, 2022), the potency data analysis from Remington *et al.* (2020) and Houben *et al.* (2020) showed an **adequate quantity of data** available for dose-distribution modelling and an **adequate potential for biases** with the available data for fish regarding the allocation of data points along the threshold distribution and the fact that the population study was almost equally composed of children and adults from eight countries in Europe and from the United States of America. There were no data available for regions in Asia, Africa or South America where fish represent an important part of the diet. In the case of 35 individuals challenged with multiple species of fish (cod, salmon, catfish and mackerel), data from only the most sensitive results were included in dose-distribution modelling.

Among the 82 data points, there were 5 left censored (6 percent) and 10 right censored (12 percent). Seventy-eight were obtained from published literature and four from unpublished studies. Among these four adults (unpublished data), the first dose tested was high at 890 mg with not surprisingly two left-censored data points. Among the 78 clinical data points remaining, the lowest dose tested reported left -censoring at 46.1 mg and two patients reacted to this first dose with objective symptoms among a population of nine patients with positive OFC (Helbling *et al.*, 1999).

The ED_{01} for fish from the overall dataset (combination of fish species) was established at 2.6 mg ($CI_{95\%}$: 1.0, 12.0) for the discrete dose dosing scheme and appears to be lower for the cumulative dose dosing scheme (1.3 mg [$CI_{95\%}$: 0.4, 12.7]) based on the Bayesian stacked model averaging methodology. This can be explained by the shape of the distribution model with a steeper slope of the curve than for the other allergens which leads to predicted discrete population EDp values that are actually slightly higher than the cumulative population EDp values. The ED_{05} was defined as 12.1 mg ($CI_{95\%}$: 4.5, 43.9) (discrete doses) with the Bayesian stacked model averaging methodology. There is no single-dose challenge study available to verify the ED_{01} and the ED_{05} for fish in an unselected outpatient clinic population.

6.5 CRUSTACEA (ALL MEMBERS OF SUBPHYLUM *CRUSTACEA*)

Shrimps, prawns, crabs and lobsters are of main interest as allergenic foods in the category of crustacean products. Allergy to crustaceans mostly affects the adult population, but children can also be affected (Lao-araya and Trakultivakorn, 2012; Sasaki *et al.*, 2018; Osterballe *et al.*, 2005). For a crustacean-allergic individual, the probability of reacting to another crustacean species has been estimated to be 75 percent (Torres Borrego, Martínez Cuevas and Tejero García, 2003). The major allergen from crustacean shellfish is tropomyosin, a muscle protein, although several other allergenic proteins including arginine kinase and myosin light chain have been identified in shrimp and other crustacean shellfish as well as molluscan shellfish (Lopata, O'Hehir and Lehrer, 2010; Bauermeister *et al.*, 2011; Pascal *et al.*, 2015; Johnston *et al.*, 2019).

There is evidence that crustacean food allergy is more prevalent in Asia, Australia and parts of Europe such as Spain where crustacean seafood is more widely consumed (see section on prevalence from Part I of this Ad hoc Joint FAO/WHO Expert Consultation). The available data for dose-distribution modelling only related to shrimp and multiple species of shrimp are present in the dataset. A major data gap exists as to whether a threshold dose for shrimp can be extended to other crustaceans such as crab or lobster. The only food challenge data available for crab provided a positive response at 19 g of crab protein (Atkins, Steinberg and Metcalfe, 1985). To the best of our knowledge there are no food challenge data available for molluscan shellfish.

AVAILABLE/ACCESSIBLE STUDIES

As detailed in the supplementary information from Remington *et al.* (2020), there are four studies available for shrimp (three from published literature and one unpublished clinical dataset) with a total of 75 individuals included in the analysis (0 left-censored, 38 right-censored); 73 identified as adults, and two identified as children.

QUALITY/QUANTITY

In Part 1 of this Ad hoc Joint FAO/WHO Expert Consultation, the analysis of the potency data from Remington *et al.* (2020) and Houben *et al.* (2020) showed an **adequate quantity of data** available for dose-distribution modelling (n = 75). A **high potential for biases** with the available data for shrimp was attributed because the population study was mainly composed of adults (n = 73) with only two children. This can be considered a data gap since shrimp allergy can affect both adults and children. Furthermore, food challenge data came from five countries in two regions (Europe and North America), and similarly to fish there was no data from Southeast Asia where prevalence of shrimp allergy is clearly documented. Additionally, a limited number of species of shrimp and no other crustacean species have been used in these studies.

Among the 75 data points, there were no left censored and 38 right censored (51 percent). Fifty-two data points were obtained from published literature and 23 from unpublished studies. This large proportion of right censored data may indicate that the allocation of data points along the threshold distribution is not well-balanced, in part due to the unexpectedly high individual thresholds in the shrimp-allergic population (Ballmer-Weber *et al.*, 2015). For three out of four studies, the first dose tested was very low (below 0.03 mg) explaining why there are no left censored data, but for one study the first dose tested was relatively high (912 mg proteins), and no patient (n = 21) was reported to react at this dose. This result would support a high ED_{01} and ED_{05} for shrimp compared to the other allergenic foods, as reported in several studies.

The ED_{01} (discrete dosing scheme) was established at 26.2 mg protein ($CI_{95\%}$: 2.7, 166) and the ED_{05} at 280 mg proteins ($CI_{95\%}$: 29.3, 880) for shrimp with the Bayesian stacked model averaging methodology. Large confidence intervals for both EDps reflect the uncertainties around those estimates for this allergenic food. There is no single-dose challenge study available to verify the ED_{01} and the ED_{05} for shrimp in an unselected outpatient clinic population. These predicted eliciting doses represent the best approximation for other crustaceans until the data gap can be filled.

6.6 SESAME SEED (*SESAMUM INDICUM*)

Sesame seed allergy seems to persist for life, similar to allergies to fish or peanuts (Agne *et al.*, 2004). Cohen *et al.* reported that 20 percent of 74 sesame seed-allergic paediatric patients in Israel developed tolerance during the follow-up period of 1.8–14 years (median 6.4 years) (Cohen *et al.*, 2007). Sesame seed allergy appears to be present more frequently during childhood and notably in infants and young children under three years of age (Garkaby *et al.*, 2021; Peters *et al.*, 2017; see also the first meeting report of this Expert Consultation on prevalence of food allergy), although onset may occur at any age (Dalal, Goldberg and Katz, 2012). Sesame seed allergy is also associated with tree-nut and peanut allergies (Brough *et al.*, 2020).

Most of the proteins present in sesame seeds are storage proteins composed of globulins (67.3 percent), albumins (8.6 percent), prolamins (1.4 percent) and glutelins

(6.9 percent) (Poveda *et al.*, 2016; Wang *et al.*, 2020). The major allergen is the 2S albumin Ses i 1, which is thought to contribute to the allergenicity of sesame seed and the association of peanut, tree nut and sesame seed allergies (Dreskin *et al.*, 2021). Other allergens include the 11S seed storage globulin, also thought to play a role in cross-reactive allergies with walnut (Wallowitz *et al.*, 2007) while oleosins have been identified as minor allergens (Elhers *et al.*, 2019).

Both sesame seeds (flour and paste) and sesame seed oil have been reported to cause allergic reactions (Kanny, De Hauteclocque and Moneret-Vautrin, 1996; Sokol *et al.*, 2020). Sesame seed oil extracted by mechanical pressure (cold), a method appreciated by the consumer from a taste point of view, is considered to be lightly or not refined. Cold-pressed sesame seed oil contains more proteins than it would if it were highly refined (Crevel *et al.*, 2000). The extraction method, which differs from one production of sesame seed oil to another, could explain the variation in allergenicity (Agne *et al.*, 2004). This is the reason why manifestations of immediate food allergy have been reported to sesame seed oil and then confirmed by positive oral challenge tests. Additionally, anaphylactic shock has been reported after consuming foods cooked with sesame seed oil (Kanny, De Hauteclocque and Moneret-Vautrin, 1996).

AVAILABLE/ACCESSIBLE STUDIES

As detailed in the supplementary information from Remington *et al.* (2020), at the time of the March 2021 meeting, four studies were available for sesame seed (three from published literature and one unpublished clinical dataset) with a total of 40 individuals included in the analysis (3 left censored, 10 right censored); 18 identified as adults, and 20 identified as children. In addition to the data from Remington *et al.* (2020) and Houben *et al.* (2020), one study was identified for consideration for sesame seed (see Annex 1 – Studies considered from potency subgroup review).

When originally reviewing the datasets for sesame seed during the second meeting in March 2021, the expert committee identified the existence of significant datasets which had not yet been included in the dose-distribution modelling. These datasets were obtained and analysed and the results discussed at an additional plenary meeting of the expert committee in March 2022. As a result, the data for sesame seed finally comprised 246 data points across 11 studies (Turner *et al.*, 2022c). Of these, five DBPCFC studies provided 67 (including five data points from an unpublished study), while six open challenge studies provided 179. Of these observations, 57 were left-censored and 10 right-censored.

QUALITY/QUANTITY

The data quality and quantity conclusions reached in Part 1 of this Ad hoc Joint FAO/WHO Expert Consultation were updated based on the enlarged dataset with quantity being classed as "**good**" and quality as "**adequate potential for biases**". While the study population is almost equally composed of children and adults, data from DBPCFC (n = 67) were available from only three countries (France,

the Netherlands and the United States of America) covering two Codex regions. However, inclusion of open challenge data extends coverage to seven countries and three Codex regions, including some where a higher prevalence 0.4–0.7 percent of the general population than other parts of the world is noted.

Turner *et al.* (2022c) updated the dose-distribution analysis for sesame in an analysis incorporating the totality of available studies. The discrete and cumulative ED_{01} values were 0.2 mg sesame seed protein (95 percent CI 0.09–1.0 and 0.08–1.0 respectively) while ED_{05} values were 2.4 (95 percent CI 1.0–7.7) and 2.5 (95 percent CI 0.9–9.5) mg of sesame seed protein, respectively. These ED estimates did not significantly change when sensitivity analyses were performed which excluded data from unblinded food challenges. In discussion over derivation of an RfD for sesame seed, a member of the expert committee expressed concerns over inclusion of a high number of studies based on open challenges, reducing the number of DBPCFC observations to 67. The specific concern was that some of those studies had relatively high starting doses, leading to an underestimate of the RfD. It was pointed out that, in fact, Turner *et al.* (2022c) had also investigated both of these concerns and found that including these studies with a relatively large number of left censored data points was more protective rather than less protective as their inclusion reduced the modelled ED_{05} (and ED_{01}) values. An additional analysis that excluded all studies using open challenges did not significantly alter the ED_{05} value, although unsurprisingly it considerably increased the 95 percent confidence interval around that value.

On the basis of these data and analyses, the expert committee recommended use of the ED_{05} value based on the enlarged dataset as a basis for the sesame reference dose as it provided the most conservative starting point from the available sesame seed datasets.

There is no single-dose challenge study available to verify the ED_{01} and the ED_{05} for sesame seed in an unselected population.

6.7 HAZELNUT (*CORYLUS AVELLANA*)

The clinical presentation of hazelnut allergy varies from mild symptoms limited to the oropharynx (oral allergy syndrome, OAS) to potentially life-threatening anaphylaxis. The frequency and the type of hazelnut-induced allergic reactions seem to vary considerably by geographic region and are related to the geographical distribution of inhaled cross-reactive pollens (birch/hazel trees) (Tang, 2018).

Primary hazelnut allergy, frequently characterized by generalized systemic and often severe reactions is due to immunoglobulin E (IgE) against specific major hazelnut allergens (notably the 11S seed storage globulin Cor a 9 and the 2S albumin Cor a 14) (Datema *et al.*, 2015) and is more prevalent in children younger than five years old (Calamelli *et al.*, 2021). OAS (or pollen food syndrome) is the result of cross-reactivity between homologous proteins contained in both pollens (notably birch pollen) and certain plant-derived foods including hazelnut. OAS is typically seen in adolescents and adults with a history of seasonal allergic rhinitis and rarely leads to anaphylaxis (Calamelli *et al.*, 2021).

Hazelnuts represent the main cause of tree nut allergy in Northern Europe in adults and school-age children (Lyons *et al.*, 2019, 2020) and prevalence section of the first report (FAO and WHO, 2022) explaining why many individual threshold data points have been reported from this region.

As with other tree nuts, resolution of hazelnut allergy is considered infrequent (Fleischer *et al.*, 2005). Regarding cross-reactivity, walnut, pecan and hazelnut form a group of strongly cross-reactive tree nuts (Goetz, Whisman and Goetz, 2005). For example, the European Pronuts study showed that 74 percent of the children with hazelnut allergy were allergic to walnut, and 56 percent of children with walnut allergy also had a hazelnut allergy (Brough *et al.*, 2020).

AVAILABLE/ACCESSIBLE STUDIES

As detailed in the supplementary information from Remington *et al.* (2020), there are ten studies available for hazelnut (eight from published literature and two unpublished clinical datasets) with a total of 411 individuals included in the analysis (9 left-censored, 205 right-censored); 248 identified as adults, and 163 identified as children. In addition to the data from Remington *et al.* (2020) and Houben *et al.* (2020), three studies were identified for consideration for hazelnut (see Annex 1–Studies considered from potency subgroup review).

QUALITY/QUANTITY

In Part 1 of this Ad hoc Joint FAO/WHO Expert Consultation (FAO and WHO, 2022), analysis of the potency data from Remington *et al.* (2020) and Houben *et al.* (2020) showed a **good quantity of data** available for dose-distribution modelling (n = 411) and an **adequate potential for biases** with the available data for hazelnut.

An *adequate* potential for biases with the available data for hazelnut was attributed because 1) the number of individual thresholds available is high, and 2) the population study was composed of adults (n = 248) and children (n = 163) in a proportion that would support a representative sample of the hazelnut-allergic population. However, although well-distributed, data were available from countries in Europe only where hazelnut allergy is the more prevalent tree nut allergy.

Among the 411 clinical data points, there were 9 left-censored and 205 right-censored (corresponding to 50 percent of the dataset). This high amount of right-censored data may indicate that the allocation of data points along the threshold distribution is not well balanced. However, the inclusion of the birch-pollen related hazelnut-allergic individuals, who may have a higher reactivity threshold, could have shifted the results to the right part of the dose-response curve. Lastly, it is noted that a large proportion (one-third) of the dataset included unpublished data.

From published data, the first dose tested was often very low (below 0.03 mg) explaining why there are a low number of left-censored data points, but for three studies where the first dose tested was between 1 and 1.7 mg protein, three patients (one in each study) were reported to react at these doses (i.e. 1 mg, 1.6 mg and 1.7 mg).

The ED_{01} (discrete dosing scheme) was established at 0.1 mg protein ($CI_{95\%}$: 0.07, 0.6) and the ED_{05} at 3.5 mg protein ($CI_{95\%}$: 1.3, 12.1) for hazelnuts with the Bayesian stacked model averaging methodology. Relatively large confidence intervals for both EDps can be attributed to the important role of the right-censored data. There is no single-dose challenge study available to verify the ED_{01} and the ED_{05} for hazelnuts.

6.8 CASHEW NUTS (*ANACARDIUM OCCIDENTALE*)

Despite the lack of data reported in unselected populations, the prevalence of cashew nut allergy varies from region to region and seems to be particularly of concern in Europe, Australia and the United States of America (McWilliam *et al.*, 2015 and prevalence section of the first report [FAO and WHO, 2022]). In terms of clinical presentation, the reported symptoms of cashew nut allergy are commonly classified as severe and potentially life-threatening (Mendes *et al.*, 2019). The cashew nut as well as the pistachio nut belong to the *Anacardiaceae* family and are thus botanically closely related. A high degree of serological cross-reactivity has been demonstrated between cashew nut and pistachio by sIgE- inhibition tests (van der Valk *et al.*, 2014). This serological cross-reactivity translates into clinical reactivity. Thus, the European Pronuts study reported that almost all children (97 percent) with pistachio allergy were allergic to cashew, and 83 percent of children allergic to cashew were allergic to pistachio (Brough *et al.*, 2020).

AVAILABLE/ACCESSIBLE STUDIES

As detailed in the supplementary information from Remington *et al.* (2020), there are three studies available for cashew (two from published literature and one unpublished clinical dataset) with a total of 245 individuals included in the analysis (16 left censored, 112 right censored); none identified as adults, and 244 identified as children. In addition to the data from Remington *et al.* (2020) and Houben *et al.* (2020), three studies were identified for consideration for cashew (see Annex 1–Studies considered from potency subgroup review).

QUALITY/QUANTITY

In Part 1 of this Ad hoc Joint FAO/WHO Expert Consultation, analysis of the potency data from Remington *et al.* (2020) and Houben *et al.* (2020) showed a **good quantity of data** available for dose-distribution modelling (n = 245) and a **high potential for biases** with the available data for cashew.

The *high* potential for biases with the available data for cashew nuts was attributed because 1) the population study was composed of children exclusively even though cashew allergy similar to the other tree nuts allergies has a low resolution rate and usually persists into adulthood; 2) all the available threshold data were only collected in one country in Europe (the Netherlands); and 3) among the 245 clinical data points, there were 16 left censored and 112 right censored (corresponding to 46 percent of the dataset).

In addition, for cashew nuts, a high percentage (31 percent) of the available data were issued from unpublished data. Regarding the two published studies, the lowest dose tested was 1 mg of cashew nut protein and seven children (in a cohort of 136 patients) reacted at this first dose.

The ED_{01} (discrete dosing scheme) has been established at 0.05 mg protein ($CI_{95\%}$: 0.02, 0.3) and the ED_{05} at 0.8 mg proteins ($CI_{95\%}$: 0.2, 5) for cashew nuts with the Bayesian stacked model averaging methodology. Despite a good quantity of data available for dose-distribution modelling, the confidence intervals for both EDps are relatively wide, and this may be attributed to the large proportion of right-censored data. Because of the close botanical relationship and the sequence homology between their major allergens, cashew and pistachio EDps are assumed to be similar. However, no specific individual threshold data exist for pistachio nuts. There is no single-dose challenge study available to verify the ED_{01} and the ED_{05} for cashew nuts in an unselected outpatient clinic population.

6.9 WALNUT (*JUGLANS REGIA*)

Walnut is an important elicitor of food allergy in children and adults with a high rate of severe reactions (Ballmer-Weber *et al.*, 2019). Walnut and pecan are closely related botanically with the allergens having a high level of sequence identity and similarity (Smeekens, Bagley and Kulis, 2018). Allergies to the two tree nuts are similarly closely allied and have been shown through the multicentre Pronuts study in Europe to be co-existent. The Pronuts study reported that almost all (97 percent) children with pecan allergy were allergic to walnut, but only 75 percent of children allergic to walnut were allergic to pecan (Brough *et al.*, 2020). Walnuts also cross-react with hazelnuts (see above). An important walnut allergen is the 2S albumin, Jug r 1, one of the first allergenic 2S albumins to be identified which, like 2S albumins from other tree nuts and seeds is thought to play an important role in clinical cross-reactivity (Dreskin *et al.*, 2021). Other important allergens include the seed storage globulins Jug r 2, r 4 and 6 and the Bet v 1 homologue Jug r 5 which show similar patterns of sensitization across Europe to hazelnut (Lyons *et al.*, 2021). An Israeli study with 56 walnut-allergic individuals reported that 82 percent were co-allergic to pecan, 27 percent were co-allergic to hazelnut and 34 percent were co-allergic to cashew (Elizur *et al.*, 2019). When comparing the median values of individual minimum eliciting doses (MEDs) for walnut and pecan, walnut was significantly lower than pecan (210 mg [50–465 mg] vs 540 mg [125–1250 mg], median [IQR], respectively) (Elizur *et al.*, 2019). A second, larger study from the same group also reported that the median MEDs were significantly lower in walnut versus pecan challenges (80 mg [40–210 mg] vs 180 mg [100–660 mg], median [IQR], respectively) (Goldberg *et al.*, 2021).

These data suggest that, while there are no data for pecan, the application of the walnut data to pecan could be overly precautionary. However, it should be noted that in this same study, the pecan challenges yielded a significantly greater number of patients with several severe clinical manifestations (such as systemic skin reactions, lower respiratory symptoms, and treatment with bronchodilators) compared with walnut challenges (Goldberg *et al.*, 2021).

AVAILABLE/ACCESSIBLE STUDIES

As detailed in the supplementary information from Remington *et al.* (2020), there are two studies available for walnut (one from published literature and one unpublished clinical dataset) with a total of 74 individuals included in the analysis (5 left-censored, 31 right-censored); 33 identified as adults, and 41 identified as children. In addition to the data from Remington *et al.* (2020) and Houben *et al.* (2020), one study was identified for consideration for walnut (see Annex 1–Studies considered from potency subgroup review).

QUALITY/QUANTITY

In Part 1 of this Ad hoc Joint FAO/WHO Expert Consultation, analysis of the potency data from Remington *et al.* (2020) and Houben *et al.* (2020) showed an **adequate quantity of data** available for dose-distribution modelling (n = 74) and a **high potential for biases** with the available data for walnut.

The *high* potential for biases with the available data for walnut was attributed essentially because all the available threshold data were only collected in one country in Europe (the Netherlands). Unpublished iFAAM study (pending publication) data would report similar results on walnuts as those from Remington *et al.* (2020) and Houben *et al.* (2020), but the high potential for bias remains in the available walnut data because the iFAAM study was also conducted in Europe. Additionally, it is noted that a high percentage (55 percent) of the available walnut data were issued from unpublished data.

Among the 74 clinical data points, 5 were left-censored and 31 right-censored (corresponding to 42 percent of the dataset). The first dose tested in the published dataset was very low (0.03 mg) explaining why there were no left-censored data in the published study (Blankestijn *et al.*, 2017). For the unpublished study, while the first dose tested was 1.05 mg protein, 5 out of 41 children were left-censored and had their reactivity thresholds below this dose.

The ED_{01} (discrete dosing scheme) was established at 0.03 mg protein ($CI_{95\%}$: 0.01, 0.5) and the ED_{05} at 0.8 mg protein ($CI_{95\%}$: 0.1, 8.9) for walnuts with the Bayesian stacked model averaging methodology. Relatively large confidence intervals for both EDps can be partially attributed to the high proportion of right-censored data. There is no single-dose challenge study available to verify the ED_{01} and the ED_{05} for walnuts in an unselected outpatient clinic population.

6.10 ALMOND (*PRUNUS DULCIS*)

No ED data have been published for almond. Furthermore, almonds are not closely related botanically to any of the other tree nuts. Thus, EDp values are not proposed for almond.

6.11 EGGS (HEN'S EGG)

In the Codex Alimentarius glossary pertaining to veterinary drug residues (CAC/MISC 5-1993), egg refers to the "fresh edible portion of the spheroid body produced by female birds, especially domestic fowl" (FAO and WHO, 2003). However, all food challenge data refer to hen's egg (i.e. *Gallus gallus*). Although hen's egg allergy is among the most common food allergies in infants and young children, it is usually considered to have a good prognosis for later life because of the high rates of resolution. Resolution rates vary among studies, probably owing to differences in patient selection and methods used to assess egg allergy (Foong and Santos, 2021). In a retrospective review in North America, approximately 40 percent and 70 percent of egg-allergic children with clear clinical history of an IgE-mediated allergic reaction to egg had developed tolerance to concentrated egg at 10 and 16 years of age, respectively (Savage *et al.*, 2007). The majority of allergenic proteins are contained in egg white (four major allergens) rather than egg yolk (two major allergens). Indeed, analysis of oral food challenge data indicates that pasteurized egg white is more potent than whole egg in causing allergic reactions (Allen *et al.*, 2014).

The majority of egg-allergic children (65–81 percent) can tolerate egg in a baked product such as muffins or cookies. This is because extensive heating during the baking process reduces allergenicity of some proteins (by destroying conformational epitopes) and reduces access to the allergen by interaction with the food matrix (Tan *et al.*, 2013).

AVAILABLE/ACCESSIBLE STUDIES

As detailed in the supplementary information from Remington *et al.* (2020), there are 21 studies available for hen's egg (18 from published literature and three unpublished clinical datasets) with a total of 431 individuals included in the analysis (52 left censored, 47 right censored); ten identified as adults, and 401 identified as children. In addition to the data from Remington *et al.* (2020) and Houben *et al.* (2020), 14 studies were identified for consideration for egg (see Annex 1–Studies considered from potency subgroup review).

QUALITY/QUANTITY

In Part 1 of this Ad hoc Joint FAO/WHO Expert Consultation, analysis of the potency data from Remington *et al.* (2020) and Houben *et al.* (2020) showed a **good quantity of data** available for dose-distribution modelling (n = 431) and a **low potential for biases** with the available data for hen's egg.

A *low* potential for biases with the available data for eggs was attributed because 1) the number of individual thresholds available is high, and 2) the population study was essentially composed of children (n = 401) in a proportion that would support a representative sample of the overall egg-allergic population. However, while the data were available from a high number of countries, it originated only in two regions of the world, Europe (including Turkey) and North America.

Among the 431 clinical data points, the dosing scheme reported 52 left-censored (12 percent) and 47 right-censored (11 percent) individuals indicating that the allocation of data points along the threshold distribution (upper-, medium- and lower-end of the distribution) was balanced. Published data from Australia (Peters *et al.*, 2014) have not been included in the dataset because the labial challenges were conducted as part of the dosing scheme, and the amount of egg protein in these labial challenges was difficult to quantify and, in some cases, may have been larger than the first ingested dose. This factor would have biased the dose-distribution modelling for eggs.

Ninety-two data points were obtained from unpublished studies (representing 21 percent of the dataset). Among these 92 patients (unpublished data), the lowest first dose tested was 0.014 mg. At this amount of allergen, one child was declared as left-censored indicating that he/she would have a reactivity threshold lower than this amount of egg protein. Among the 339 clinical data points remaining (published data), the lowest dose tested reported as left-censored was 0.53 mg and four patients reacted to this first dose with objective symptoms among a population of 20 patients with positive OFC (Morisset *et al.*, 2003).

It is also important to indicate that, to avoid overestimating the eliciting doses, the challenge materials recorded in the database relate to the raw/pasteurized, lightly cooked and powdered forms of egg and exclude results obtained with the egg incorporated into a baked form (e.g. muffins, cakes).

The ED_{01} (discrete dosing scheme) was established at 0.2 mg protein ($CI_{95\%}$: 0.1, 0.5) and the ED_{05} at 2.3 mg protein ($CI_{95\%}$: 1.2, 4.7) for eggs with the Bayesian stacked model averaging methodology. All of the existing threshold data arise from challenges with hen's egg (chicken egg). No threshold data exist for eggs from other species of birds, but cross-reactivity is well known to occur (Langeland, 1983).

There is no single-dose challenge study yet available to verify the ED_{01} and the ED_{05} for hen's egg in an unselected outpatient clinic population with a sufficient degree of statistical rigour.

6.12 COW'S MILK (*BOS TAURUS*)

In the Codex Alimentarius glossary pertaining to veterinary drug residues (CAC/MISC 5-1993), milk is "the normal mammary secretion of milking animals obtained from one or more milkings without either addition to it or extraction from it, intended for consumption as liquid milk or for further processing" (FAO and WHO, 2003).

Cow's milk allergy (CMA) is present mainly in children. IgE-mediated CMA can develop from the neonatal period after introduction of cow's milk in the diet. Different phenotypes of cow's milk allergies exist, with some phenotypes resolving earlier, some tolerating baked forms of the allergen, and some persisting into late adolescence and adulthood. Studies indicate that approximately 50–70 percent of patients achieve tolerance within three to five years (Foong and Santos, 2021).

Brand and Rick-van Gent (2011) stated that 75 percent of Dutch infants with CMA are cow's milk tolerant by the age of one year and 90 percent by the age of four years. A prospective study conducted in the United States of America showed that CMA resolved in 53 percent of subjects at a median age of 5.3 years in a cohort of 293 children aged 3 to 15 months at baseline (Wood *et al.*, 2013). As with eggs, cooking reduces the allergenicity of cow's milk by destroying many conformational epitopes (Venter *et al.*, 2017) and, depending on studies, 60–75 percent of children become tolerant to baked/heated forms of cow's milk (such as muffin and waffles) before they become tolerant to pure/uncooked forms of cow's milk (Nowak-Wegrzyn *et al.*, 2008; Kim *et al.*, 2011).

AVAILABLE/ACCESSIBLE STUDIES

As detailed in the supplementary information from Remington *et al.* (2020), there are 21 studies available for milk (19 from published literature and two unpublished clinical datasets) with a total of 450 individuals included in the analysis (96 left-censored, 27 right-censored); 18 identified as adults, and 429 identified as children. In addition to the data from Remington *et al.* (2020) and Houben *et al.* (2020), 15 studies were identified for consideration for cow's milk (see Annex 1).

QUALITY/QUANTITY

In Part 1 of this Ad hoc Joint FAO/WHO Expert Consultation, analysis of the potency data from Remington *et al.* (2020) and Houben *et al.* (2020) indicated a **good quantity of data** available for dose-distribution modelling (n = 450) and a **low potential for biases** with the available data for cow's milk.

The attribution of a low potential for biases with the available data for milk was based on 1) the high number of individual thresholds available; 2) the population study which was essentially composed of children (n = 429) in a proportion that would support a representative sample of the overall milk allergic population; and 3) the data available from a high number of countries in several regions of the world, Europe (including Turkey), Australia, South and North America.

Among the 450 clinical data points, the dosing scheme reported 96 left-censored (21 percent) and 27 right-censored (6 percent). This proportion of left-censored data is larger than other datasets in part due to the relatively high starting doses in some early clinical challenge protocols (e.g. 1 or 5 ml cow's milk, 33 or 165 mg cow's milk protein respectively), and may indicate that the allocation of data points along the threshold distribution (upper-, medium- and lower-end of the distribution) is not well balanced.

Sixty-eight data points were obtained from unpublished studies (representing 15 percent of the dataset). Among these 68 patients (unpublished data), the lowest first dose tested for children was 1.75 mg. At this level of allergen, one child was declared left censored indicating that he/she would have a reactivity threshold lower than this amount of milk protein. The only available data for

adults were obtained from an unpublished source. The first dose tested for adults was 0.35 mg, and at this dose five patients were declared as left-censored. Among the remaining 382 clinical data points (published data), the lowest left censored dose was 0.17 mg and nine children reacted to this first dose with objective symptoms among a population of 60 patients with positive OFC (Longo *et al.*, 2008).

It is noted that the challenge materials used in the challenges represented in the database were not the baked form of milk. This was done to avoid overestimating eliciting doses. In addition, no differences were found regarding reactivity thresholds between liquid milk and non-fat dried milk (skimmed milk powder) that were used in the different protocols to derive EDps (Allen *et al.*, 2014).

The ED_{01} (discrete dosing scheme) was established at 0.2 mg protein ($CI_{95\%}$: 0.1, 0.5) and the ED_{05} at 2.4 mg protein ($CI_{95\%}$: 1.3, 5.0) for cow's milk with the Bayesian stacked model averaging methodology.

For cow's milk, a single-dose challenge study to validate a predicted ED_{05} of 0.5 mg protein was recently published (Turner *et al.*, 2021). In this multicentre study, 172 children (median age six years old, 57 percent male) were included in the analysis. Twelve (7 percent) children experienced objective symptoms and were considered allergic according to predetermined criteria. Of those, one patient had mild anaphylaxis in response to a single-dose of adrenaline/epinephrine, and the remainder experienced only mild symptoms that required no pharmacological treatment. With 7 percent of children reacting to the 0.5 mg dose of milk protein, the ED_{05} for cow's milk protein was validated to be at or around 0.5 mg of milk protein. This validated ED_{05} is lower than the ED_{05} established at 2.4 mg of cow's milk protein from FARRP/TNO (Remington *et al.*, 2020; Houben *et al.*, 2020). The reasons for this difference are not entirely clear, but the selection of the patient population may have contributed.

The Turner *et al.* (2021a) study raised concerns among some members of the expert committee about the validity of the ED_{05} value derived from the much larger Remington *et al.* (2020) and Houben *et al.* (2020) datasets. Some members were aware that significant milk datasets which could not be included in the analysis undertaken in March 2021 were then available. In order to address these issues, the expert committee proposed to explore whether these datasets could be incorporated into the analyses, in parallel with a further review of milk severity data, prior to a decision on the reference dose for milk. These datasets were obtained and analysed, and the results were discussed at the additional plenary March 2022 meeting of the expert committee.

Blom *et al.* (2022) updated the dose-distribution of cow's milk protein challenge data, adding 247 data points from two studies (Turner *et al.*, 2022b; Yanagida *et al.*, 2017) to the 450 data points already reported by Remington *et al.* (2020) and Houben *et al.* (2020) giving a total of 697 data points. Dose-distribution modelling indicated discrete and cumulative ED_{01} values respectively of 0.3

(95 percent CI 0.2–0.7) and 0.4 (95 percent CI 0.3–0.9), and respective ED_{05} values of 3.2 (95 percent CI 1.8–6.4) and 4.4 (95 percent CI 2.4–9.0). These values represented small changes (well within the confidence intervals) compared to the values obtained from the original distribution. A sensitivity analysis was undertaken examining the influence of data from another large study (Rolinck-Werninghaus *et al.*, 2012) which did not meet the criteria for inclusion in the new dose-distribution modelling. Despite the additional 305 data points, the ED_{01} and ED_{05} values did not significantly change from the Houben *et al.* (2020) estimates, testifying to the robustness of the original estimates.

The single-dose challenge (Turner *et al.*, 2021) which gave rise to the original concerns about the validity of the proposed ED_{05} value was also re-examined as it contributed 83 participants to the updated reproducibility analysis. Of the 12 who had reacted to 0.5 mg, only four were included in the updated analysis, the remaining eight originated from a single centre and were younger (median age ten months, IQR 6–12 months), suggesting a higher sensitivity in that age group. This finding was supported by unpublished data from the Europrevall study showing that children <3.5 years old had consistently (and considerably) lower ED_{10} values than children >3.5 years old. From an allergy management perspective, infants represent a lower concern as they are relatively protected from severe outcomes and their dietary intake is easier to control.

Taking into account the ED_{05} from the Houben *et al.* (2020) paper, as well as the updated population dose-distribution and the sensitivity analysis, the expert committee recommended using the ED_{05} from Houben *et al.* (2020) as the basis of the cow's milk protein reference dose. The expert committee noted that the data suggested that infants appeared to be more sensitive than older children. However, given that this group is relatively protected from severe outcomes of cow's milk allergy and that intake is easier to control in that group, the expert committee considered that a reference dose based on an ED_{05} derived from the whole population dose-distribution was appropriate.

Members of the expert committee noted that the level of protection from reactions proposed through the use of a reference dose derived from the ED_{05} was superior to the level generally mandated by regulatory standards for hydrolysed infant formula preparations. These generally follow the guidance set out by the American Academy of Pediatrics. This level of protection requires demonstration with 95 percent confidence that 90 percent of the sensitive population will not react adversely (American Academy of Pediatrics, 2000). This standard was in force until recently in the European Union (Directive 2006/141/EC). This instrument has now been replaced by Commission Delegated Regulation (European Union) 2016/127, which now points to guidance by the European Food Safety Authority in relation to such protein hydrolysates. However, the guidance itself (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2021) focuses on the need to demonstrate efficacy in reducing the risk of developing allergy to milk proteins, rather than on the capacity of those hydrolysates to provoke reactions in milk-allergic infants.

6.13 PEANUT (*ARACHIS HYPOGEA*)

Peanut allergy is primarily an IgE-mediated allergy and is one of the most common food allergies in countries with a western lifestyle, notably in North America, Australia and the United Kingdom of Great Britain and Northern Ireland (see prevalence section of first report [FAO and WHO, 2022]). Although about 10–20 percent of peanut allergic individuals will outgrow this allergy, the majority of patients remain allergic for life (Skolnick *et al.*, 2001). However, peanut allergy may also recur after resolution, and a recurrence rate of approximately 8 percent was determined in patients who outgrew their peanut allergy (Fleischer *et al.*, 2005).

In western countries for which extensive data are available, peanut allergy is associated with higher rates of accidental exposure, severe reactions, and life-threatening anaphylaxis compared to other food allergies. About 7 to 14 percent of people with peanut allergy experience accidental exposure to peanuts each year, and one-third to one-half of those may experience anaphylaxis, although different definitions of anaphylaxis are used (Lieberman *et al.*, 2021).

Peanut can be consumed boiled or roasted, crushed or ground, or as an oil, a paste (peanut butter), or a flour. The wide uses of peanuts and derived products in processed foods in some regions make unintended exposure frequent.

AVAILABLE/ACCESSIBLE STUDIES

As detailed in the supplementary information from Remington *et al.* (2020), there are 27 studies available for peanut (23 from published literature and four unpublished clinical datasets) with a total of 1 306 individuals included in the analysis (61 left censored, 275 right censored); 160 identified as adults, and 1 079 identified as children. In addition to the data from Remington *et al.* (2020) and Houben *et al.* (2020), 22 studies were identified for consideration for peanut (see Annex 1).

QUALITY/QUANTITY

In Part 1 of this Ad hoc Joint FAO/WHO Expert Consultation (FAO and WHO, 2022), analysis of the potency data from Remington *et al.* (2020) and Houben *et al.* (2020) indicated a **good quantity of data** available for dose-distribution modelling and an **adequate potential for biases** with the available data for peanut.

The peanut dataset is the most robust of the priority allergen datasets due to its number of observations, distribution of thresholds across the dosing spectrum, and acquisition of data from multiple centres.

Despite the good quantity of data available for peanuts (n = 1 306), the potential for biases with the available data was only qualified as *adequate* because 1) the study population was mostly composed of children (n = 1 079) in a proportion that may not be a representative sample of the overall peanut allergic population considering that an important percentage of peanut-allergic children will keep their allergy in adulthood; and 2) most of the data were available from a limited number of countries

in a limited number of regions in the world, essentially the United Kingdom in Europe and the United States of America in North America for published data, and from the Netherlands for unpublished data.

Among the 1 306 clinical data points, the results of the dosing scheme included 61 left-censored (5 percent) and 275 right-censored (21 percent) individuals indicating that the allocation of data points along the threshold distribution (upper-, medium- and lower-end of the distribution) was balanced.

Four hundred and fifty-seven data points were obtained from unpublished studies (representing 35 percent of the dataset). Among these patients (unpublished data), the lowest first dose tested for children was 0.005 mg. At this level of allergen, two children were declared as left-censored indicating that they would have a reactivity threshold lower than this amount of peanut protein. Among the remaining 849 clinical data points (published data), the lowest dose tested reported left-censored at 0.003 mg, and one child reacted to this first dose with objective symptoms among a population of 43 patients with positive OFC (Ballmer-Weber *et al.*, 2015).

For peanuts, the ED_{01} (discrete dosing scheme) was established at 0.2 mg protein ($CI_{95\%}$: 0.1, 0.4) and the ED_{05} at 2.1 mg protein ($CI_{95\%}$: 1.2, 4.6) with the Bayesian stacked model averaging methodology.

Several other studies have published EDp values for objective symptoms during food challenges to peanuts (Klemans *et al.*, 2015; Ballmer-Weber *et al.*, 2015; Blumchen *et al.*, 2014; Blom *et al.*, 2013; Eller, Hansen and Bindslev-Jensen, 2012) in which the values obtained for ED_{01} and ED_{05} were of the same order of magnitude as for the 2020 Remington/Houben studies, and many of these are included in the combined dataset analysis by the 2020 Remington/Houben studies. In a similar approach, i.e. based on a retrospective analysis of published data from oral food challenges studies, Zhu *et al.* (2015) derived population thresholds based on a literature review, although the methodology has been criticized as not involving discussion of severity scoring with the authors of the original studies. The minimum eliciting doses for severe reactions to peanut were significantly higher than for mild or moderate reactions (Zhu *et al.*, 2015). In Zhu *et al.* (2015), the estimated ED_{10} values for peanut allergic individuals were some of the lowest values reported in the literature based on a modelling approach. Differences were likely due to the fact that Zhu *et al.* (2015) included patients with subjective symptoms (such as OAS) who were not included in many other studies and not included in the 2020 Remington/Houben studies. However, it should be noted that similarly low estimates were seen when others investigated subjective symptoms (Klemans *et al.*, 2015; Ballmer-Weber *et al.*, 2015) or a combination of subjective and objective responses (Ballmer-Weber *et al.*, 2015). Moreover, Zhu *et al.* (2015) excluded from their analysis all the right-censored individuals which could affect the EDp estimates and skew them to be more sensitive. Lastly, the total number of patients included in the Zhu *et al.* (2015) analysis was much lower than the total number of patients challenged with peanuts in the relevant studies, introducing a potential selection bias. In the United States of America multicentre study, Haber *et al.* (2021) estimated the dose-response distribution for peanut allergen using data from 548 DBPCFCs (n = 481 subjects,

including 67 with repeat challenges) by testing a population that was largely made up of people recruited for OIT with inclusion criteria that limited participants to those with reactive DBPCFC thresholds below certain amounts (Haber *et al.*, 2021). For example, ~25 percent of the participants included in the Haber *et al.* (2021) analysis are from the POISED study, which investigated sustained outcomes in oral immunotherapy for peanut allergy and required a positive result from a DBPCFC to ≤500 mg peanut protein to be included in the study (Chinthrajah *et al.*, 2019). According to the results published by Houben *et al.* (2020), these inclusion criteria would remove ~35 percent of the peanut allergic population from being eligible for the clinical trial and thus not eligible to be included in the Haber *et al.* (2021) analysis. Bayesian model averaging was considered, but these authors considered that the Weibull model dominated so strongly that model averaging was not needed. The ED_{01} and ED_{05} (95 percent CI) were 0.052 (0.021, 0.13) and 0.49 (0.22, 0.97) mg peanut protein, respectively, almost four times lower than in the Remington/Houben studies. However, if a subset of Houben *et al.* (2020) dataset was made to exclude diagnostic datasets and only analyse studies identified as immunotherapy studies, similar results are found as to those by Haber *et al.* (2021) indicating a potential significant selection bias in the study by Haber *et al.* (2021). Selective exclusion of such a large proportion of the peanut allergic population conflicts with the approach of modelling the overall threshold distribution for establishing a dose that is expected to elicit symptoms in a specified proportion of the whole peanut allergic population. Therefore, the dataset used in the Remington/Houben studies provides a more reliable basis for establishing the HBGV.

A single-dose challenge to validate a predicted ED_{05} established at 1.5 mg peanut protein was published in 2017 (Hourihane *et al.*, 2017). The results of this study (conducted across three centres in the United States of America, Ireland and Australia) supported "the safety of the statistically determined ED_{05} based on dose-distribution modelling for an administration to a non-selected patient population" (Hourihane *et al.*, 2017). Among the 378 children (54 percent male), eight subjects (2.1 percent; 95 percent CI = 0.6 percent–3.4 percent) met the predetermined criteria for an objective and likely related event. No child experienced more than a mild reaction, four of the eight received oral antihistamines only as part of clinical centre policy, and none received epinephrine.

The 1.5 mg dose of peanut protein is lower than the ED_{05} value established at 2.1 mg peanut protein obtained from the FARRP/TNO dataset (Remington *et al.*, 2020; Houben *et al.*, 2020), but at this dose (1.5 mg), 2.1 percent of 378 patients experienced allergic reactions instead of the 5 percent expected. Hence, this result would confirm the relevance of an ED_{05} established at 2.1 mg peanut protein.

6.14 SUMMARY

The outputs from the plenary review and discussion of the ED_{01} and ED_{05} values derived are summarized in the Table 2 (data sources and methods: Remington *et al.*, 2020; Houben *et al.*, 2020; Westerhout *et al.*, 2019; Wheeler *et al.*, 2021). These form the values for which hazard characterization was performed, with consideration

for the nature and characteristics of reactions observed among those responding to those amounts of protein from the priority allergenic foods.

While the table lists the complete set of discrete and cumulative ED_{01} and ED_{05} values, the hazard characterization group followed the rule used in deriving RfDs in the VITAL™ Program, namely, to use the lowest discrete or cumulative relevant EDp value for each allergenic food.

TABLE 2 **FOOD-ALLERGIC POPULATION ELICITING DOSES (EDS)**

	DISCRETE ED_{01} (95% CI)	CUMULATIVE ED_{01} (95% CI)	DISCRETE ED_{05} (95% CI)	CUMULATIVE ED_{05} (95% CI)
CASHEW	0.05 (0.02, 0.3)	0.09 (0.04, 0.5)	0.8 (0.2, 5.0)	1.6 (0.4, 9.4)
EGG	0.2 (0.1, 0.5)	0.2 (0.1, 0.5)	2.3 (1.2, 4.7)	2.4 (1.3, 5.3)
FISH	2.6 (1.0, 12.0)	1.3 (0.4, 12.7)	12.1 (4.5, 43.9)	15.6 (4.6, 102)
HAZELNUT	0.1 (0.07, 0.6)	0.2 (0.09, 0.7)	3.5 (1.3, 12.1)	4.7 (1.7, 15.7)
MILK	0.2 (0.1, 0.5)	0.3 (0.2, 0.6)	2.4 (1.3, 5.0)	3.1 (1.6, 6.6)
Blom *et al.* (2022)	0.3 (0.2, 0.7)	0.4 (0.3, 0.9)	3.2 (1.8, 6.4)	4.3 (2.4, 9.0)
PEANUT	0.2 (0.1, 0.4)	0.7 (0.5, 1.3)	2.1 (1.2, 4.6)	3.9 (2.8, 7.1)
SESAME	0.1 (0.03, 2.7)	0.2 (0.04, 4.8)	2.7 (0.4, 33.6)	4.2 (0.6, 57.7)
Turner *et al.* (2022c)	0.2 (0,09, 1.0)	0.2 (0.08, 1.0)	2.4 (1.0, 7.7)	2.5 (0.9, 9.5)
SHRIMP	26.2 (2.7, 166)	30.8 (3.4, 326)	280 (69.3, 880)	429 (94.0, 1854)
WALNUT	0.03 (0.01, 0.5)	0.04 (0.02, 0.6)	0.8 (0.1, 8.9)	1.2 (0.1, 13.0)
WHEAT	0.7 (0.3, 2.5)	1.1 (0.4, 3.8)	6.1 (2.6, 15.6)	9.3 (3.9, 24.9)

Source: Reproduced from Remington *et al.* (2020) unless otherwise noted. **Remington, B.C., Westerhout, J., Meima, M.Y., Blom, W.M., Kruizinga, A.G., Wheeler, M.W., Taylor, S.L., Houben, G.F. & Baumert, J.L.** 2020. Updated population minimal eliciting dose-distributions for use in risk assessment of 14 priority food allergens. *Food and Chemical Toxicology*, 139: 111259. https://doi.org/10.1016/j.fct.2020.111259

CHAPTER 7
DETAILED HAZARD CHARACTERIZATION AT POTENTIAL REFERENCE DOSES

7.1 INTRODUCTION AND BASIC PRINCIPLES

In terms of IgE-mediated food allergy, "severity" is a complex, multidimensional construct which is influenced by exposure dose and route as well as cofactors such as age, comorbidity and exercise (Turner *et al.*, 2016). These issues impact upon the choice of hazard characterization in terms of protecting the food-allergic population from "severe" reactions.

Under current legislation in the European Union (European Union, 2002), food may be considered "unsafe" if it is injurious to health, for example, due to the "particular health sensitivities of a specific category of consumers" such as those with food allergies. However, what precisely constitutes "injurious to health" is not explicitly defined; indeed, interpretation of the law indicates that provided a food product is labelled in accordance with legal requirements (i.e. including priority allergenic ingredients where appropriate), food is safe, unless it is specifically marketed for people with those health sensitivities. Commission guidance states specifically:

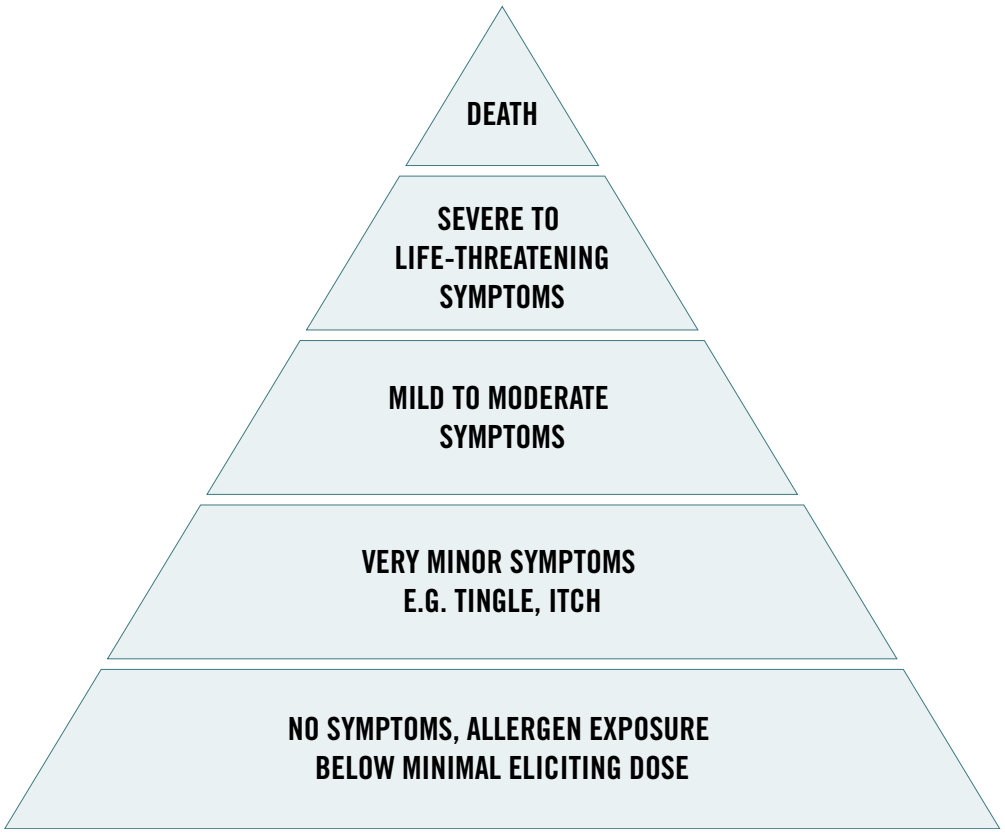
> Article 14 (4) (c) of Regulation (EC) No 178/2002 requires that if food is produced for a group of consumers with particular health sensitivities (e.g. intolerant or allergic), then these sensitivities should be taken into account when determining whether a food is injurious to health. An example would be food that is unintentionally cross contaminated with nuts, which would be injurious to health if it was designed for those who needed a nut-free diet. However, when a product is not making a claim that it is intended for a group with particular health sensitivities, the fact that it may be harmful for

> that group does not automatically mean it is injurious within the meaning described in this Regulation (except where the mandatory information is not appropriately communicated) (European Union, 2010).

In Canada, food is also considered to be unsafe if it contains undeclared food allergens, whether as an ingredient or an unintended presence due to shared production facilities (Canada, 1985); however, the requirement for allergen declaration "does not apply to a food allergen or gluten that is present in a pre-packaged product as a result of cross-contamination" (Canada, 2021).

The Food Allergen Labelling and Consumer Protection Act (2004) (FALCPA) in the United States of America more explicitly enshrines the concept of an "allergic response that causes a risk to human health," which implies that some reactions do not pose such a risk (Dubois *et al.*, 2018). By definition, therefore, there is a hierarchy of risks faced by people susceptible to food allergy, some of which might not be considered to be a risk to human health (Figure 2).

FIGURE 2. **HIERARCHY OF RISKS FACED BY PEOPLE SUSCEPTIBLE TO IgE-MEDIATED FOOD ALLERGY**



Source: Reproduced with permission from Dubois *et al.*, 2018. **Dubois, A.E.J., Turner, P.J., Hourihane, J., Ballmer-Weber, B., Beyer, K., Chan, C.-H., Gowland, M.H. *et al.*** 2018. How does dose impact on the severity of food-induced allergic reactions, and can this improve risk assessment for allergenic foods?: Report from an ILSI Europe Food Allergy Task Force Expert Group and Workshop. *Allergy*, 73(7): 1383–1392. https://doi.org/10.1111/all.13405

As explained by Dubois *et al.* (2018):

> severity is a highly subjective term which stakeholders use and interpret in different ways. Some symptoms may be visually severe (such as rash, facial swelling), without involving respiratory or cardiovascular compromise. Others (e.g. impaired cognition, fluctuating consciousness and subtle abnormalities in cardiac output) are potentially life threatening, but may not appear significant to nonhealthcare professionals or laypersons. Indeed, non-expert clinicians in ambulatory settings, lacking familiarity with the diversity of generalized allergic reactions, may also over or underestimate reaction severity (Dubois *et al.*, 2018, p. 1385–1386).

Fatal food anaphylaxis is the most extreme harm that can occur, but fortunately, it is a very rare event, occurring at less than 1 per 100 000 person years in food-allergic individuals (Table 3) (Umasunthar *et al.*, 2015). Investigating fatal reactions is extremely difficult: It is usually impossible to accurately determine the amount of allergen that has been consumed or the presence of other factors which might have contributed to the fatal outcome (although to date, there are no reports of fatal reactions to levels of exposure not exceeding the ED_{05} for any allergenic food). Furthermore, the vast majority of reported fatal anaphylaxis cases are due to the inadvertent consumption of an allergen either intentionally or unintentionally found in non-prepacked foods (Turner *et al.*, 2015, 2017); these foods are unlikely to have had a PAL statement such as is used with prepacked foods. The rarity of fatal reactions and their limited relevance in the context of managing unintended allergen presence makes fatal reactions an inappropriate basis for characterizing the hazard posed by such presence (Turner *et al.*, 2022a). Therefore, the expert committee concluded that fatal anaphylaxis was not a useful measure in terms of characterizing hazard at potential reference doses.

TABLE 3 **SUMMARY OF ESTIMATED FOOD ANAPHYLAXIS RATES FOR FOOD-ALLERGIC PEOPLE**

ANAPHYLAXIS DEFINITION	AGE GROUP	ESTIMATED ANNUAL INCIDENCE RATE IN FOOD-ALLERGIC POPULATION
Self-reported food anaphylaxis	All ages	Less than 1 episode every **10** person years
	Aged 0–19	Less than 1 episode every **10** person years
Medically coded food anaphylaxis	All ages	Less than 1 episode every **300** person years
	Aged 0–19	Less than 1 episode every **250** person years
	Aged 0–4	Less than 1 episode every **5** person years
Hospital admissions for food anaphylaxis	All ages	Less than 1 episode every **1 000** person years
	Aged 0–19	Less than 1 episode every **2 000** person years
	Aged 0–4	Less than 1 episode every **1 000** person years
Fatal food anaphylaxis	All ages	Less than 1 episode every **100 000** years
	Aged 0–19	Less than 1 episode every **100 000** years

Source: Adapted from Umasunthar *et al.*, 2015. **Umasunthar, T., Leonardi-Bee, J., Turner, P.J., Hodes, M., Gore, C., Warner, J.O. & Boyle, R.J.** 2015. Incidence of food anaphylaxis in people with food allergy: a systematic review and meta-analysis. *Clinical and Experimental Allergy*, 45(11): 1621–1636. https:// doi.org/10.1111/cea.12477

It is therefore reasonable to consider non-fatal, severe allergic reactions in hazard characterization. In the context of establishing reference doses to protect the allergic individual, the relationship between dose and severity is poorly defined:

> The only modifiable parameter, which may be controlled by public health measures for food allergy, is exposure to the allergen, i.e. dose; while limiting exposure is known to decrease the rates of reactions in allergic populations, the impact of this on the relative frequency of severe reactions at different doses is unclear (Dubois *et al*., 2018, p. 1390).

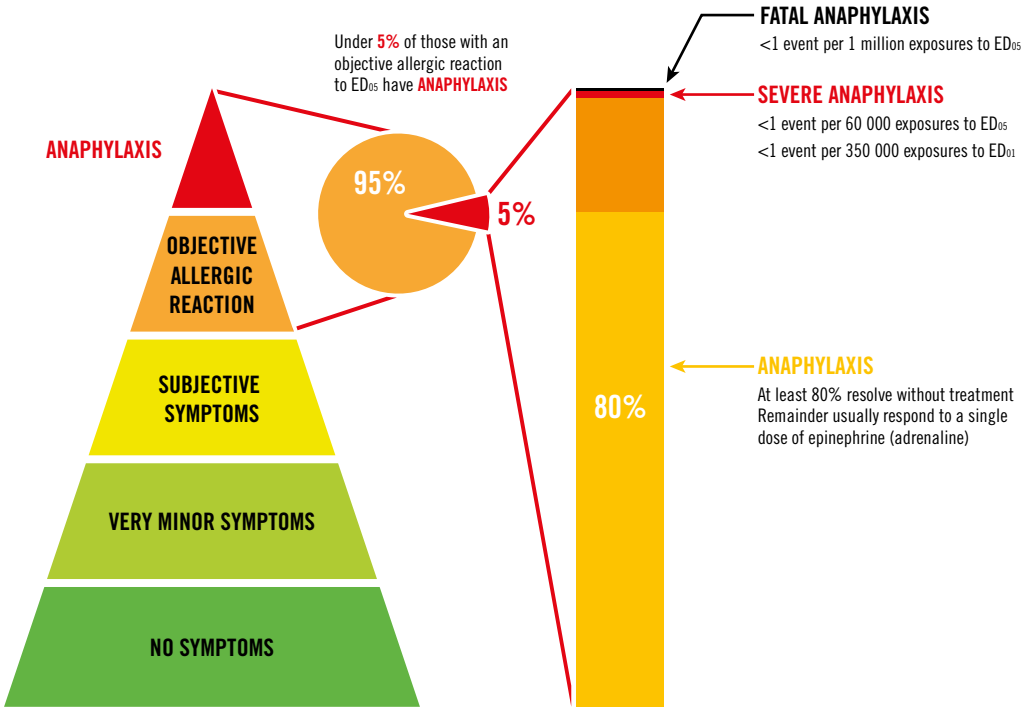
because reaction severity "is dependent on multiple factors and variables, some of which are plausibly still unknown" (Dubois *et al.*, 2018). At a population level, dose appears to have a very limited role in determining severity of allergic reaction. Furthermore, severity of prior reaction does not predict future severity, nor is anaphylaxis reproducible for a given level of allergen exposure (Patel *et al.*, 2021a). Given these uncertainties, the experts concluded that any attempts at hazard characterization must rely on actual data (rather than, for example, modelling approaches) to assess severity.

However, the assignment of severity for food-induced allergic reactions is inconsistent in the literature, and each method has its limitations (Stafford *et al.*, 2021). There is no universally-accepted system for scoring the severity of food-allergic reactions, but most clinicians would consider reactions involving airway/breathing and/or cardiovascular compromise as severe (Turner *et al.*, 2022a). The experts therefore agreed to use "anaphylaxis" as the definition of severity for hazard characterization. Even though there are multiple definitions of anaphylaxis in the literature, there is international consensus that allergic reactions involving airway/breathing and/or cardiovascular compromise constitute "anaphylaxis."

Nonetheless, even non-fatal anaphylaxis is not a single entity in terms of severity. Turner *et al.* (2022a) recently summarized the available evidence (Figure 3). At least 80 percent of anaphylaxis reactions are not treated with epinephrine/adrenaline but nonetheless resolve spontaneously (even though this practice is contrary to international guidelines). This demonstrates the spectrum of severity for anaphylaxis, from mild reactions which spontaneously resolve to more severe reactions which are refractory to initial treatment; the latter occur in 3.4 percent (95 percent CI 1.9–5.9 percent) of epinephrine-treated reactions (Patel *et al.*, 2021b). In those reporting anaphylaxis to any level of exposure for a food allergen, the risk of fatal outcome is estimated to be <1:10 000 (Umasunthar *et al.*, 2013); it is likely that this rate would be even lower following a low-dose exposure such as at an ED_{05} level. Therefore, the expected rate of fatal reaction to an ED_{05} exposure in an allergic individual can be estimated to be <1 per million (Figure 3). There are currently no reports in the literature of fatal reactions to this level of exposure, for any allergenic food.

The experts therefore agreed that in terms of hazard characterization, the objective was to **minimize, to a point where further refinement does not meaningfully reduce public health impact, the probability of any clinically relevant**

FIGURE 3. **HIERARCHY OF RISKS FACED BY PEOPLE SUSCEPTIBLE TO IGE-MEDIATED FOOD ALLERGY, PROPORTIONATE TO THEIR ESTIMATED OCCURRENCE FOR PEANUT IN PEANUT-ALLERGIC INDIVIDUALS**



Source: Reproduced with permission from Turner *et al.*, 2022a.

Note: ED_{01}, the eliciting dose predicted to provoke reactions in 1% of the allergic population; ED05, the eliciting dose predicted to provoke reactions in 5% of the allergic population. **Turner, P.J., Patel, N., Ballmer-Weber, B.K., Baumert, J.L., Blom, W.M., Brooke-Taylor, S., Brough, H. *et al.*** 2022a. Peanut can be used as a reference allergen for hazard characterization in food allergen risk management: a rapid evidence assessment and meta-analysis. *The Journal of Allergy and Clinical Immunology: In Practice*, 10(1): 59–70. https:// doi.org/10.1016/j.jaip.2021.08.008

objective allergic response following exposure to the unintended presence of allergens:

> defined by dose-distributions relating to objective allergic symptoms observed in up to XX percent of the relevant allergic population (EDp); and

> supported by data regarding **severity of symptoms** in likely range of envisioned thresholds (mg protein), as defined by occurrence of anaphylaxis reactions up to any given level of exposure (envisioned RfD) with additional consideration to non-anaphylaxis symptoms that might be experienced by a proportion of the allergic population at that level of exposure.

The approach adopted by the experts was therefore to assess the likelihood of allergic symptoms (including anaphylaxis) to peanut at low-doses of exposure (up to approximately the ED_{05} 95 percent confidence interval upper bound), given that this allergen has the largest evidence base. The experts would then evaluate whether allergic reactions to peanut can be considered a "worst-case" scenario by assessing the available evidence for other priority allergens.

Factors which would be considered in undertaking this evaluation would include:

> the proportion of reactions occurring at potential reference doses which would be defined as anaphylaxis;

> the reproducibility of these data, i.e. what proportion of allergic individuals not reacting to a level of allergen exposure equivalent to a potential reference dose might react to an equivalent exposure on another occasion, and with anaphylaxis;

> the nature of non-anaphylaxis symptoms which might be experienced at potential reference doses, and by what proportion of the food-allergic population;

> the applicability of data obtained at double-blind, placebo-controlled food challenges to "real world" allergen exposure; and

> the impact of cofactors such as exercise, stress, concomitant medication and so forth on reaction severity.

7.2 PEANUT

A recent updated analysis for the ED_{01} and ED_{05} for peanut using the joint TNO/FARRP database reported the following values (Remington *et al.*, 2020) in Table 4.

TABLE 4 **FOOD-ALLERGIC POPULATION ELICITING DOSES (EDS) FOR PEANUT**

	ED_{01}		ED_{05}	
	DISCRETE	CUMULATIVE	DISCRETE	CUMULATIVE
PEANUT	0.2mg (0.1, 0.4)	0.7mg (0.5, 1.3)	2.1mg (1.2, 4.6)	3.9mg (2.8, 7.1)

Source: Adapted from Remington *et al.*, 2020. **Remington, B.C., Westerhout, J., Meima, M.Y., Blom, W.M., Kruizinga, A.G., Wheeler, M.W., Taylor, S.L., Houben, G.F. & Baumert, J.L.** 2020. Updated population minimal eliciting dose-distributions for use in risk assessment of 14 priority food allergens. *Food and Chemical Toxicology*, 139: 111259. https://doi.org/10.1016/j.fct.2020.111259
All values mg peanut protein (95 percent confidence interval).

Patel *et al.* (2021a) undertook a systematic review of published data reporting reaction thresholds in over 3 000 peanut-allergic individuals undergoing double-blind, placebo-controlled food challenges (DBPCFC) to peanut. The analysis found that approximately 4.5 percent (95 percent CI 1.9 percent–10.1 percent) (see also Table 10) of individuals reacting to ≤5 mg peanut protein with objective symptoms will experience anaphylaxis. For an exposure to ≤1 mg peanut protein, 4.2 percent (95 percent CI 0.7 percent–22.3 percent) of individuals with objective symptoms will have anaphylaxis. The ≤1 mg cut-off used approximates to the upper limit of the 95 percent confidence interval for ED_{01} to peanut, while the ≤5 mg cut-off approximates to the upper limit of the 95 percent confidence interval for the ED_{05}. On this basis, the authors estimated that anaphylaxis occurs to an ED_{05} level of peanut exposure in 2.3 (95 percent CI 1.0–5.0) individuals per 1 000 with peanut allergy, and 0.4 (95 percent CI 0.1–2.2) individuals per 1 000 in those exposed to an ED_{01} amount. Thus, use of ED_{01} or ED_{05} would in both cases be associated with a rate of up to 5 percent of individuals with objective symptoms to that level of exposure developing symptoms consistent with anaphylaxis (Table 5).

The experts identified three reports in the literature with respect to subjective symptoms experienced as a result of low-dose exposures to peanut at food challenge. In the Peanut Allergen Threshold Study (PATS), 378 peanut-allergic children underwent a single-dose challenge to 1.5 mg peanut protein; 67 (17.7 percent; 95 percent CI 14–22 percent) developed subjective symptoms (Hourihane *et al.*, 2017). In addition, both Blom *et al.* (2013) and Ballmer-Weber *et al.* (2015) report threshold dose-distribution curves for any symptom (subjective + objective) in addition to objective symptoms. The latter report also identified that at cumulative doses of 0.33–3.33 mg peanut protein, around 5–10 percent of peanut-allergic individuals will experience mild transient symptoms of oral allergy syndrome (OAS) (Patel *et al.*, 2021a). The experts were therefore able to make the following determination for the likelihood of allergic symptoms to the following levels of peanut exposure in the peanut-allergic population:

TABLE 5 **PROPORTION OF PEANUT-ALLERGIC INDIVIDUALS WHO WOULD BE EXPECTED TO HAVE SYMPTOMS FOLLOWING AN EXPOSURE TO AN ED05 OR ED01 AMOUNT OF PEANUT. OAS, ORAL ALLERGY SYMPTOMS. ESTIMATES OF THE OCCURRENCE OF DIFFERENT SYMPTOMS ARE BASED ON THE LITERATURE**

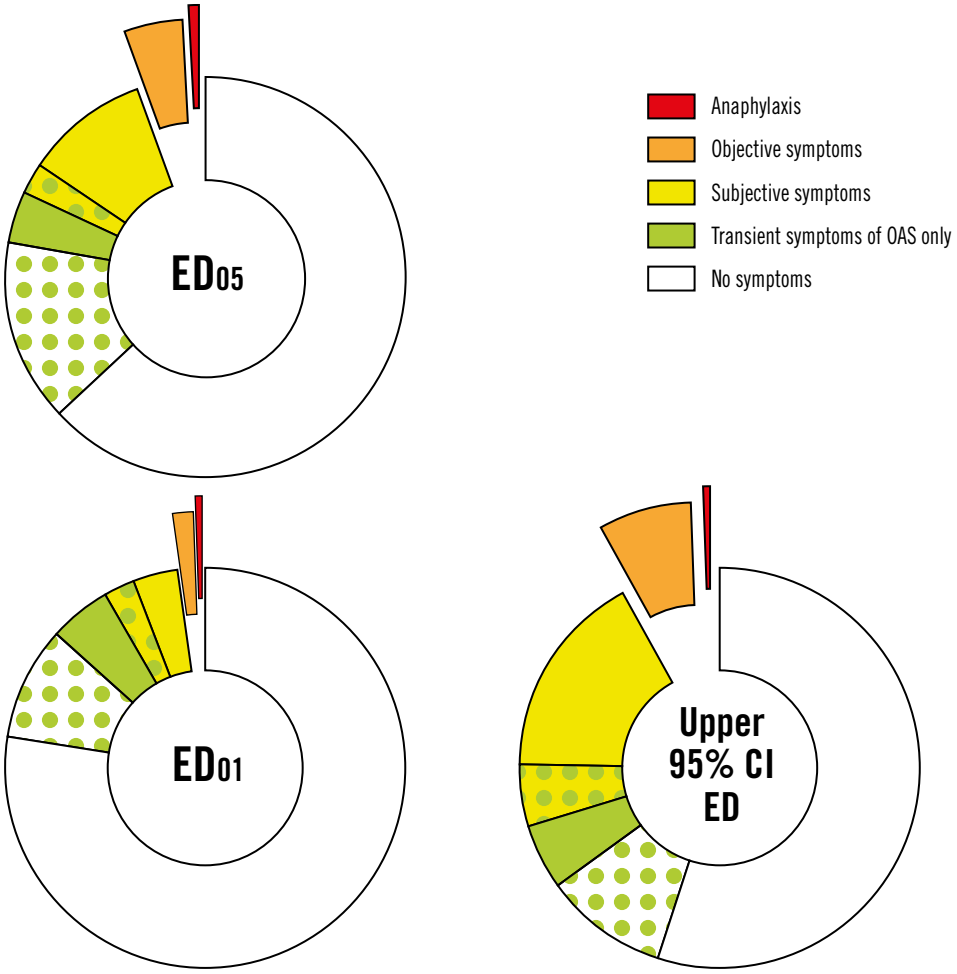
	PROBABILITY OF SYMPTOMS FOLLOWING AN EXPOSURE TO		
PEANUT	1 MG PROTEIN (≈upper 95%CI for cum ED_{01})	2.1 MG PROTEIN (discrete ED_{05})	7.1 MG PROTEIN (upper 95%CI for cumED_{05})
ANY SYMPTOM (subjective or objective)	14%[1] to 23%[2]	20%[1] to 35%2	35%[1] to 45%[2]
SUBJECTIVE SYMPTOMS	13%[1] to 22%[2]	15% to 30%[1-3]	27%[1]to 37%[2]
OAS ONLY	5% to 10%[2]	5% to 10%[2]	5% to 10%[2]
ANY OBJECTIVE SYMPTOM (based on ED_{01}/ED_{05} definition)	1%	5%	8%[5]
ANAPHYLAXIS RATE:			
- in those reacting to **this dose** with objective symptoms	4.2%[4] (95%CI 0.7–22.3%)	4.5%[4] (95%CI 1.9–10.1%)	
- overall, in the peanut-allergic population	0.04%[4] (95%CI 0.01–0.22%)	0.23%[4] (95%CI 0.1–0.5%)	

Source: Adapted with permission from Turner *et al.*, 2022a. **Turner, P.J., Patel, N., Ballmer-Weber, B.K., Baumert, J.L., Blom, W.M., Brooke- Taylor, S., Brough, H. *et al.*** 2022a. Peanut can be used as a reference allergen for hazard characterization in food allergen risk management: a rapid evidence assessment and meta-analysis. *The Journal of Allergy and Clinical Immunology: In Practice*, 10(1): 59-70. https:// doi.org/10.1016/j.jaip.2021.08.008

Note: Some data from the above table are from: 1) Blom *et al.*, 2013; 2) McWilliam *et al.*, 2020; 3) van der Valk *et al.*, 2016; 4) Ballmer-Weber *et al.*, 2015; and 5) Houben *et al.*, 2020.

Patel *et al.* (2021a) also undertook an individual patient data (IPD) meta-analysis of 534 peanut allergic individuals who had undergone at least two DBPCFCs to peanut on separate occasions. This allowed for an assessment of the reproducibility of the reaction threshold (and the occurrence of anaphylaxis) over time. They found that individual thresholds could vary by up to three logs, although in the majority (71 percent), this variation was limited to a half-log change in eliciting dose. Overall, 2.4 percent (95 percent CI, 1.1 percent to 5.0 percent) of patients who tolerated 5 mg of peanut protein on one occasion reacted to this dose at a subsequent exposure, but none developed anaphylaxis. These data are summarized in Figure 5.

FIGURE 4. **PROPORTION OF PEANUT-ALLERGIC INDIVIDUALS EXPECTED TO HAVE SUBJECTIVE OR OBJECTIVE SYMPTOMS FOLLOWING EXPOSURE TO AN ED_{05} OR ED_{01} AMOUNT OF PEANUT. DATA FROM TABLE 5. (*OAS, ORAL ALLERGY SYMPTOMS)**



Source: Adapted with permission from Turner *et al.*, 2022a.

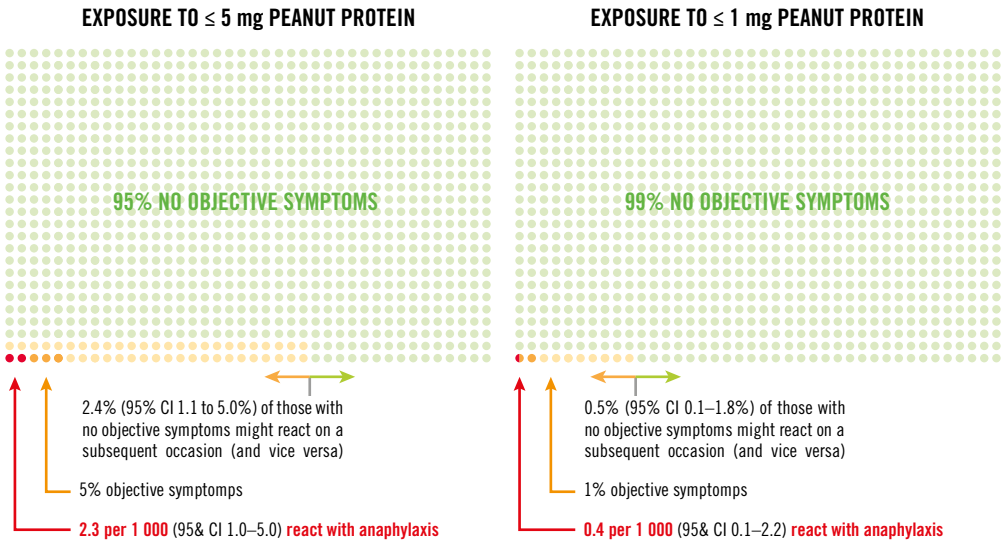
Note: OAS = oral allergy symptoms.

Note: ED_{01}, the eliciting dose predicted to provoke reactions in 1% of the allergic population; ED05, the eliciting dose predicted to provoke reactions in 5% of the allergic population. **Turner, P.J., Patel, N., Ballmer-Weber, B.K., Baumert, J.L., Blom, W.M., Brooke-Taylor, S., Brough, H. *et al.*** 2022a. Peanut can be used as a reference allergen for hazard characterization in food allergen risk management: a rapid evidence assessment and meta-analysis. *The Journal of Allergy and Clinical Immunology*: In Practice, 10(1): 59–70. https:// doi.org/10.1016/j.jaip.2021.08.008

On the basis of these data, the experts concluded that at an ED_{05} level of exposure to peanut, around one-third of peanut-allergic participants would experience subjective symptoms, the vast majority of a mild and transient nature. Among the 5 percent of individuals predicted to develop objective symptoms, only 4.5 percent of them would have anaphylaxis. This equates to 50 peanut allergic individuals per 1 000 ingesting an ED_{05} exposure dose developing objective symptoms, and 2.3 of those individuals predicted to develop anaphylaxis.

The experts then evaluated whether these estimates might differ significantly for other priority allergens.

FIGURE 5. **PROPORTION OF PEANUT-ALLERGIC INDIVIDUALS WHO WOULD BE EXPECTED TO HAVE OBJECTIVE SYMPTOMS (INCLUDING ANAPHYLAXIS) FOLLOWING EXPOSURE TO ≤5 mg OR ≤1 mg AMOUNT OF PEANUT, AND AN INDICATION OF REPRODUCIBILITY, I.E. PROPORTION OF INDIVIDUALS WHO DO NOT EXPERIENCE OBJECTIVE SYMPTOMS ON ONE OCCASION WHO MIGHT REACT ON A SECOND SUBSEQUENT EXPOSURE**



Source: Adapted with permission from Patel *et al.*, 2021a. **Patel, N., Adelman, D.C., Anagnostou, K., Baumert, J.L., Blom, W.M., Campbell, D.E., Chinthrajah, R.S. *et al.*** 2021a. Using data from food challenges to inform management of consumers with food allergy: a systematic review with individual participant data meta-analysis. *Journal of Allergy and Clinical Immunology*, 147(6): 2249–2262.e7. https://doi. org/10.1016/j.jaci.2021.01.025

7.3 TREE NUTS

The experts identified three studies in which allergic patients underwent formal food challenge (FC) to a range of tree nuts. In the Pronuts study, a multicentre European study (London, United Kingdom; Geneva, Switzerland; Valencia, Spain), 122 children (median age 5.5 years old) underwent multiple challenges to peanut, tree nut or sesame to assess co-existent allergy (Brough *et al.*, 2020). A total of 689 FCs to tree nuts were performed, of which 191 (28 percent) were positive (Table 6). Only 2 of 35 (5.7 percent) individuals had anaphylaxis to a level of exposure of ≤30 mg protein (>ED_{10}), the initial dose used for challenges.

Purington *et al.* (2018) undertook a retrospective analysis of 410 individuals median age nine years range 1–52 years) who underwent DBPCFC at seven sites in the United States of America. There were 512 positive challenges to tree nuts (almond 29, cashew 150, hazelnut 65, pecan 88, pistachio 59, walnut 120). Eliciting dose and corresponding symptom severity are shown in Figure 6. While more severe reactions were seen at all eliciting doses, there was no evidence of a higher rate of more severe reactions at lower eliciting doses compared to peanut. The relative prevalence of anaphylaxis symptoms is shown in Table 7. After peanut, cashew and pecan were associated with the highest rates of anaphylaxis symptoms. The experts therefore specifically sought additional data relating to these two tree nuts.

TABLE 6 **POSITIVE FOOD CHALLENGE (FC) IN THE PRONUTS STUDY**

	TOTAL POSITIVE FOOD CHALLENGES (N = 238)	No. REACTING TO ≤30 mg PROTEIN	ANAPHYLAXIS TO ≤30 mg PROTEIN	SYMPTOMS
ALMOND	6/69 (9%)	0/6		
BRAZIL	7/100 (7%)	0/7		
CASHEW	36/83 (43%)	10/36	0/10	
HAZELNUT	30/70 (43%)	6/30	0/6	
MACADAMIA	16/100 (16%)	3/16	1/3	Laryngeal + lower respiratory symptoms
PECAN	26/92 (28%)	5/26	0/5	
PISTACHIO	34/94 (36%)	4/34	0/4	
WALNUT	36/81 (44%)	7/36	1/7	Pruritic rash, local angioedema, stridor
PEANUT	37/66 (56%)	8/37	0/8	

Source: Adapted with permission from Brough *et al.*, 2020. **Brough, H. A., Caubet, J. C., Mazon, A., Haddad, D., Bergmann, M. M., Wassenberg, J., Panetta, V., Gourgey, R., Radulovic, S., Nieto, M., Santos, A. F., Nieto, A., Lack, G. & Eigenmann, P. A.** 2020. Defining challenge-proven coexistent nut and sesame seed allergy: a prospective multicenter European study. *The Journal of Allergy and Clinical Immunology*, 145(4): 1231–1239. https://doi.org/10.1016/j.jaci.2019.09.036

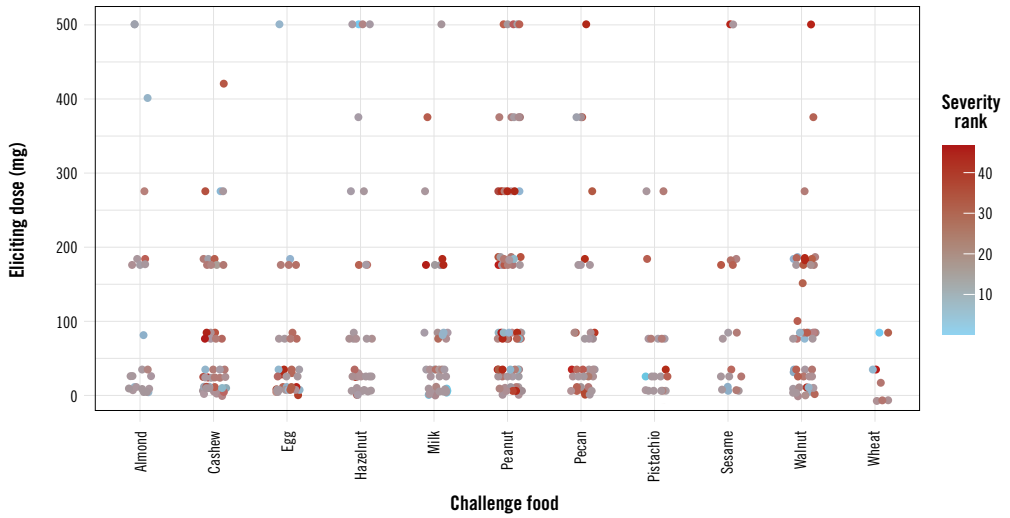
TABLE 7 **PROPORTION OF POSITIVE FOOD CHALLENGE (FC) ASSOCIATED WITH ANAPHYLAXIS**

	TOTAL POSITIVE FOOD CHALLENGES (N = 512)	AIRWAY OBSTRUCTION	WHEEZING	CARDIOVASCULAR SYMPTOMS
ALMOND	30/44 (68%)	0	0%	0
CASHEW	151/312 (48%)	1%	6.0%	1%
HAZELNUT	68/95 (72%)	0	2.9%	0
PECAN	88/165 (53%)	2%	9.1%	0
PISTACHIO	60/93 (65%)	3%	3.3%	0
WALNUT	121/195 (62%)	0	2.5%	0
PEANUT	347/795 (44%)	2.9%	8.1%	0.3%

Source: Reproduced from Purington *et al.* (2018). **Purington, N., Chinthrajah, R. S., Long, A., Sindher, S., Andorf, S., O'Laughlin, K., Woch, M. A. *et al.*** 2018. Eliciting dose and safety outcomes from a large dataset of standardized multiple food challenges. *Frontiers in Immunology*, 9: 2057. https://doi. org/10.3389/fimmu.2018.02057

In the NutCracker study, 83 patients (median age 8.7 years, range 3–24 years) were prospectively evaluated for walnut, pecan, cashew, pistachio, hazelnut, and almond allergy (Elizur *et al.*, 2018). In those without a recent clinical history, food challenges were undertaken as shown in Table 8. Although these patients did not undergo challenge to peanut, the rates of lower respiratory symptoms and/or need for rescue epinephrine due to reactions across the entire food challenge dosing range were not greater than those reported in the literature for peanut. In a subsequent publication, the same authors report 61 patients (including 31 from the original cohort) with positive food challenges to walnut (median age nine years, range 4–24 years) (Elizur *et al.*, 2020). Eleven (18 percent) experienced lower respiratory symptoms and 18 (30 percent) were treated with rescue epinephrine, only one of whom reacted to a low-level exposure (20 mg walnut protein).

FIGURE 6. **CORRELATION OF ELICITING DOSE AND ADVERSE EVENT SEVERITY RANKING BY CHALLENGE FOOD. RED RANKING CORRESPONDS TO MORE SEVERE SYMPTOMS, WHILE BLUE CORRESPONDS TO MORE MILD SYMPTOMS. ANAPHYLAXIS (AS DEFINED BY WAO, 2020) EQUATES TO A SEVERITY SCORE OF ~≥35**



Source: Reproduced with permission from Purington *et al.* (2018). **Purington, N., Chinthrajah, R. S., Long, A., Sindher, S., Andorf, S., O'Laughlin, K., Woch, M. A. *et al.*** 2018. Eliciting dose and safety outcomes from a large dataset of standardized multiple food challenges. *Frontiers in Immunology*, 9: 2057. https://doi. org/10.3389/fimmu.2018.02057

TABLE 8 **DETAILS OF POSITIVE CHALLENGES IN THE NUTCRACKER STUDY. INJECTABLE EPINEPHRINE WAS USED FOR SEVERE REACTIONS INCLUDING DIFFUSE FLUSHING, STRIDOR OR WHEEZING, SEVERE ABDOMINAL PAIN, OR REDUCED BLOOD PRESSURE AS PER THE ATTENDING PHYSICIAN'S DISCRETION**

	No. POSITIVE FOOD CHALLENGES	LOWER RESPIRATORY SYMPTOMS	USE OF RESCUE EPINEPHRINE
CASHEW	36	22%	17%
HAZELNUT	13	15%	23%
PECAN	32	19%	25%
PISTACHIO	21	19%	29%
WALNUT	45	11%	22%

Source: Adapted from Elizur *et al.*, 2018. Reproduced with permission from John Wiley and Sons. ***Elizur, A., Appel, M.Y., Nachshon, L., Levy, M.B., Epstein-Rigbi, N., Golobov, K. & Goldberg, M.R.*** 2018. NUT Co Reactivity - ACquiring knowledge for elimination recommendations (NUT CRACKER) study. *Allergy*, 73(3): 593–601. https://doi. org/10.1111/all.13353

The experts also identified a report from Australia describing 167 young people (mean age seven to eight years old) with FC-positive cashew allergy (McWilliam *et al.*, 2020). Nine (5.3 percent) had anaphylaxis at FC, the lowest eliciting dose reported for these nine reactions was 0.31 g of cashew, approximately 55 mg cashew protein.

Turner *et al.* (2022a) undertook a rapid evidence assessment to evaluate the rate of anaphylaxis to allergen exposure no greater than the upper 95th confidence interval for the ED_{05} (as reported by Houben *et al.*, 2020) for cashew, hazelnut and walnut, and performed a meta-analysis (Turner *et al.*, 2022a). The data are summarized in Table 9.

Hazelnut has been implicated as a common cause of pollen food allergy syndrome (PFAS) in Europe, due to cross-reactivity of Bet v 1 protein homologues with birch pollen (Datema *et al.*, 2018). This is also consistent with data published by Masthoff *et al.* (2018), that following low-dose exposure to hazelnut (≤10 mg protein), subjective symptoms are almost twice as common in adults (in whom PFAS is more common) than in children.

TABLE 9 **PROPORTION OF INDIVIDUALS ALLERGIC TO CASHEW, HAZELNUT AND WALNUT WHO WOULD BE EXPECTED TO HAVE SYMPTOMS FOLLOWING ORAL CONSUMPTION OF AN ED_{05} AMOUNT. ESTIMATES OF THE OCCURRENCE OF DIFFERENT SYMPTOMS ARE BASED ON THE. OAS, ORAL ALLERGY SYMPTOMS**

	CASHEW		HAZELNUT		WALNUT	
DISCRETE ED_{05}	0.8 mg		3.5 mg		0.8 mg	
CUMULATIVE ED_{05}	1.6 mg [95% CI 0.4–9.4 mg]		4.7 mg [95% CI 1.7–15.7 mg]		1.2 mg [95% CI 0.1–13.0 mg]	
	0.8 mg	9.4 mg	3.5 mg	15.7 mg	0.8 mg	13.0 mg
ANY SYMPTOM (subjective or objective)	8%[1] to >46%[2]	32%[1]	31%[1] to 50%[4]	73%[4] to 76%[1]	~8%[1]	~60%[1]
SUBJECTIVE SYMPTOMS	3%[1] to 46%[2]	20%[1] to 66%[3]	26%[1] to 48%[4]	67%[1] to 70%[4]	Not known	~46%[1]
- OAS only	11%[2]	Not known	20%–30%[4]			Not known
ANY OBJECTIVE SYMPTOM (based on ED_{05} definition)	5%	12%[5]	5%	9%[5]	5%	14%[5]
ESTIMATED RATE OF ANAPHYLAXIS:						
- in those reacting to ≤ED_{05} exposure	4.9% (95%CI 2.2–10.5%)		2.5% (95%CI 0.3–15.8%)		5.3% (95%CI 2.0–13.0%)	
- overall, in individuals with that specific food allergy	0.25% (95%CI 0.11–0.53%)		0.12% (95%CI 0.02–0.79%)		0.27% (95%CI 0.10–0.67%)	
ESTIMATES BASED ON	597 FC (318 DBPCFC, 279 Open FC)		434 FC (391 DBPCFC, 43 Open FC)		350 FC (194 DBPCFC, 156 Open FC)	

Source: Adapted from Turner *et al.*, (2022a). **Turner, P.J., Patel, N., Ballmer-Weber, B.K., Baumert, J.L., Blom, W.M., Brooke- Taylor, S., Brough, H. *et al.*** 2022a. Peanut can be used as a reference allergen for hazard characterization in food allergen risk management: a rapid evidence assessment and meta-analysis. *The Journal of Allergy and Clinical Immunology*: In Practice, 10(1): 59–70. https:// doi.org/10.1016/j.jaip.2021.08.008

Note: Some data from the above table are from 1) Blom *et al.*, 2013; 2) McWilliam *et al.*, 2020; 3) van der Valk *et al.*, 2016; 4) Ballmer-Weber *et al.*, 2015; and 5) Houben *et al.*, 2020.

Overall, the expert committee was unable to identify any evidence in the literature suggesting that tree nut-allergic individuals are more likely than peanut-allergic individuals to experience anaphlaxis due to low levels of exposure to the relevant allergen.

7.4 SESAME SEED

While sesame seed is not currently listed as a priority allergen in Codex, it is a priority allergen in the European Union, Canada, Australia and New Zealand and more recently in the United States of America. Turner *et al.* (2022a) identified nine published studies (representing 273 positive FC). A report from Sokol *et al.* (2020) was not included as this study included only three positive FC. While some

objective reactions were reported to low levels of exposure, only two (0.7 percent) anaphylaxis reactions were reported to <60 mg level exposures (equivalent to the upper 95 percent CI for ED_{05} for sesame), both occurring ~3 mg sesame protein. At meta-analysis, this was equivalent to a rate of 3.0 percent (95 percent CI: 0.8 percent to 11 percent) (see also Table 10).

It is clear that sesame seed oil – which is typically cold-pressed – can also trigger anaphylaxis in relatively small (<5 ml) volumes in some individuals (Leduc *et al.*, 2006; Dano *et al.*, 2015; Crevel *et al.*, 2000; Kanny, De Hauteclocque and Moneret-Vautrin, 1996). However, there is an absence of data with respect to the amount of protein that may be found in sesame seed oils and how it relates to eliciting doses derived from FC using sesame seeds or sesame seed flour or paste.

7.5 COW'S MILK

Blom *et al.* (2013) estimated that 13–20 percent of individuals with an allergy to cow's milk will develop subjective symptoms (subjective and/or objective) to ED_{05} levels of exposure (2.4–6.6 mg cow's milk protein). Turner *et al.* (2021) reported a single-dose challenge study in which 50 of 172 milk-allergic individuals (29 percent) developed symptoms to 0.5 mg cow's milk protein, at least 19 percent (33/172) of whom developed transient subjective symptoms, consistent with the estimate of Blom *et al.* (2013). Although cow's milk allergy is one of the most common in early childhood, the majority of children tend to outgrow it. This may explain why there is a perception that cow's milk allergy is less "serious" than other food allergies (Turner, 2013; Barnett *et al.*, 2018). In reality, there are different phenotypes, and children with persisting cow's milk allergy may be more at risk of severe reactions: indeed, cow's milk is the single most common cause of fatal anaphylaxis in children in the United Kingdom (Baseggio Conrado *et al.*, 2021a) and a common cause of fatal and near-fatal reactions elsewhere (Baseggio Conrado *et al.*, 2021b).

Turner *et al.* (2022a) identified 17 studies in the literature, representing 1 045 positive FC (98 percent in children) (Turner *et al.*, 2022a). At meta-analysis, the estimated rate of anaphylaxis in those individuals reacting with objective symptoms to ED_{05} exposure levels was 4.9 percent (95 percent CI: 2.1 percent to 11 percent) (see also Table 10).

Similar to the detailed study for peanut (Patel *et al.*, 2021a), Turner *et al.* (2022d) investigated the rate of anaphylaxis to low-dose (<5 mg cow's milk protein) controlled challenges to milk, as well as the reproducibility of minimum eliciting doses (thresholds) in study participants. At meta-analysis, 4.8 percent (95 percent CI 2.0–10.9 percent) and 4.8 percent (95 percent CI 0.7–27.1 percent) of individuals reacting to ≤5 mg and ≤0.5 mg of cow's milk (CM) protein (respectively) had anaphylaxis, equating to 0.5 and 2.4 anaphylaxis events per 1 000 patients exposed to an ED_{01} or ED_{05} dose respectively, in the broader cow's milk-allergic population. Similar results were observed for peanut (Patel *et al.*, 2021a).

Intra-individual variability in minimum eliciting dose (threshold of reaction) was investigated using data from 110 individuals from five studies who had undergone repeat challenges to <5 mg of cow's milk protein. Intra-individual variation in reaction

threshold was limited to a ½-log change in 80 percent (95 percent CI 65–89 percent) of participants. Again, this was similar to the result for peanut (Patel *et al.*, 2021a).

The experts agreed with the conclusions from the analyses that there was no evidence that cow's milk protein differed from the exemplar allergen peanut in terms of the severity of reactions to small amounts or the proportion of individuals reacting. They also agreed that reproducibility of reactions to small amounts between controlled challenges, as manifested by the extent and direction of change, did not depart from observations with peanut.

7.6 EGG

Blom *et al.* (2013) estimated that 9–14 percent of egg-allergic individuals will develop symptoms (both subjective and objective) to ED_{05} levels of exposure (Blom *et al.*, 2013). Data suggests that egg tends to cause fewer lower respiratory symptoms but more gastrointestinal symptoms compared to other allergens (Gupta *et al.*, 2015). There are only two anaphylaxis fatalities reported in the literature, one in the United States of America in a child (Sampson *et al.*, 1992) and another in the United Kingdom (in an adult, despite egg allergy being one of the most prevalent food allergies in preschool children) (Barnett *et al.*, 2018). Turner *et al.* (2022a) identified 20 studies in the literature, representing 1 180 positive FC (the vast majority – at least 9 percent – in children). At meta-analysis, the estimated rate of anaphylaxis in those individuals reacting with objective symptoms to ED_{05} exposure levels was 1.5 percent (95 percent CI: 0.02 percent to 55 percent) (see also Table 10); the wide confidence interval reflects the absence of any reported cases of anaphylaxis to ED_{05} levels of exposure in all but one of the included studies.

7.7 WHEAT

IgE-mediated wheat allergy is a relatively uncommon food allergy with a prevalence of under 0.5 percent in both children and adults (Baseggio Conrado *et al.*, 2021a); celiac disease and non-IgE-mediated wheat allergy are more common. However, near-fatal and fatal anaphylaxis have been reported (Baseggio Conrado *et al.*, 2021a; Kraft *et al.*, 2021; Cianferoni *et al.*, 2013). Furthermore, wheat anaphylaxis may be more associated with anaphylactic shock (involving cardiovascular compromise) than may other food allergens (Elizur *et al.*, 2018). Turner *et al.* (2022a) identified ten studies representing 348 positive FC (at least 90 percent in children). At meta-analysis, the rate of anaphylaxis in those individuals reacting with objective symptoms to ED_{05} exposure levels was estimated to be 2.2 percent (95 percent CI: 0.02 to 75 percent) (see also Table 10). One study, reporting the results of a multicentre trial of immunotherapy for wheat allergy, included a control group (n = 21) who underwent a further DBPCFC one year later (Nowak-Węgrzyn *et al.*, 2019). The reproducibility of eliciting dose in these individuals appears similar to that reported for peanut allergy (Umasunthar *et al.*, 2013).

Wheat is also the most common food allergen implicated in food-dependent, exercise-induced anaphylaxis (Cianferoni *et al.*, 2013). Wheat-dependent,

exercise-induced anaphylaxis (WDEIA) describes the scenario where a patient is normally tolerant to wheat but can develop symptoms (often anaphylaxis) if they exercise within two to four hours of wheat consumption (Scherf *et al.*, 2016). Data suggest WDEIA may be more prevalent in Asia (Baseggio Conrado *et al.*, 2021a; Zhu *et al.*, 2020). Exercise is not the only cofactor reported which can increase the risk of anaphylaxis in individuals who are otherwise wheat tolerant; other cofactors include aspirin and alcohol (Christensen *et al.*, 2018, 2019). It has been proposed that individuals at risk of WDEIA may be allergic to wheat but have very high reaction thresholds (see section 6.3) such that they normally tolerate wheat without symptoms in the absence of cofactors; the available literature suggests that exposure levels causing WDEIA in the presence of a relevant cofactor are well in excess of those triggering reactions in conventional IgE-mediated wheat allergy (Christensen *et al.*, 2019; Brockow *et al.*, 2015).

7.8 FISH AND SEAFOOD

Threshold data relating to fish and seafood are limited, in part because of the multiple different species of seafood globally and the surprisingly high reaction thresholds compared to other food allergens. Despite this, seafood is an emerging and important cause of anaphylaxis, including near fatal and fatal anaphylaxis globally (Baseggio Conrado *et al.*, 2021a). Data from Europrevall indicate that around one-third of allergic individuals could experience subjective symptoms to an ED_{05} level of exposure of cod or prawn/shrimp (Patel *et al.*, 2021a). In the literature review by Turner *et al.* (2022a), only three studies were identified with respect to finned fish (typically cod) and three evaluating thresholds to prawn/shrimp. With the paucity of data, no meta-analysis could be performed. Anaphylaxis has been reported to ED_{05} levels of exposure, but there was insufficient data to assess how the risk of anaphylaxis to ED_{05} levels compares to the risk for peanut (see also Table 10).

7.9 SOYBEAN

The inclusion of soya as a priority allergen in Codex is controversial, and the expert committee recently recommended its removal as a global priority allergen on the basis of a low level of prevalence and potency (FAO and WHO, 2021). Turner *et al.* (2022a) included this allergen in their analysis and identified five studies in the literature. Consistent with data suggesting that soybean is an uncommon cause of anaphylaxis globally (Baseggio Conrado *et al.*, 2021a), no cases of anaphylaxis to low (<200 mg protein) levels of exposure were identified (see also Table 10).

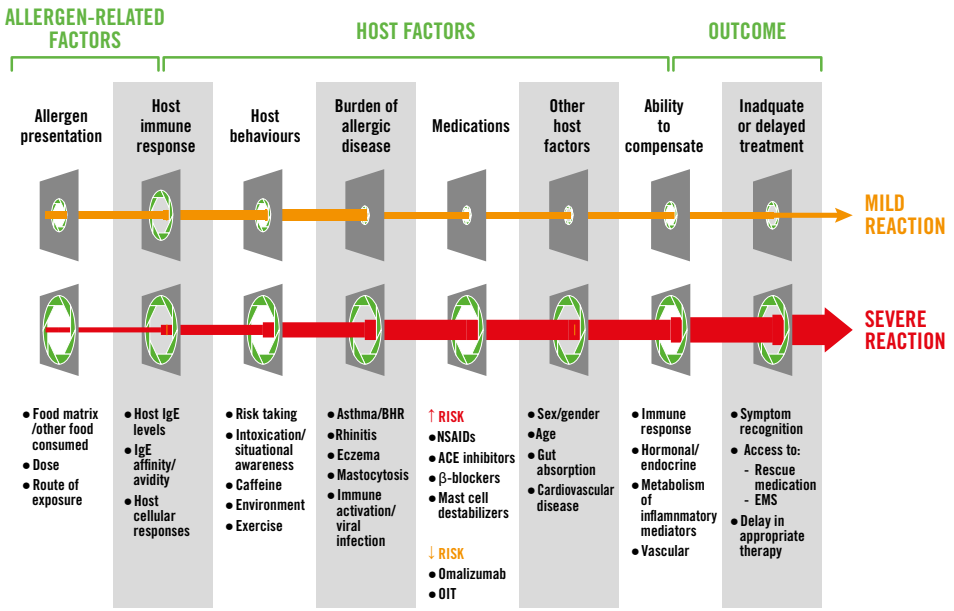
7.10 ROLE OF COFACTORS IN SEVERITY

The literature reports a number of factors which can impact the severity of food-induced allergic reactions, as outlined in Figure 7. These include cofactors or "augmentation" factors such as exercise, stress, medication and alcohol which may alter both the threshold at which individuals experience symptoms, as well as the severity of symptoms at any given level of exposure (Dubois *et al.*, 2018;

Turner *et al.*, 2016). Importantly, these cofactors are not universal: in many if not most individuals, the best-described factors (exercise, menstruation, alcohol) seem not to impact significantly on reaction severity. In a retrospective survey of almost 500 adults with food allergy, only a small proportion used medication that could influence severity, and under 10 percent reported exercise or alcohol as a relevant factor in accidental reactions (Versluis *et al.*, 2016). The authors recently published a prospective evaluation of accidental reactions in 157 patients over a one-year period. While there was a potential cofactor identified in 74 percent of reactions, no relationship was identified between the presence of these factors and reactions severity (Versluis *et al.*, 2019).

The TRACE peanut study evaluated the impact of significant exercise and sleep deprivation on peanut-induced allergic reactions in 100 peanut-allergic adults (Dua *et al.*, 2019). The authors reported a significant impact of both factors on reducing clinical thresholds by 45 percent. However, the decrease in threshold was around 0.5-log, which is well within the intra-individual variation in reaction threshold reported by Patel *et al.* 2021a. In the TRACE study, the biggest impact on variation in threshold was the clinical centre at which participants were registered. Furthermore, exercise was only identified as a significant factor in one of the two clinical centres. To date, data relating to reaction severity in the TRACE study have not been published.

FIGURE 7. **FACTORS WHICH CAN MODULATE SEVERITY OF ALLERGIC REACTIONS**



Source: Adapted with permission from Dubois *et al.* 2018.

Note: BHR, bronchial hyper responsiveness; NSAIDs, non-steroidal anti-inflammatory drugs; ACE, angiotensin-converting enzyme; EMS, emergency medical services.

Dubois, A.E.J., Turner, P.J., Hourihane, J., Ballmer-Weber, B., Beyer, K., Chan, C.-H., Gowland, M.H. *et al.* 2018. How does dose impact on the severity of food-induced allergic reactions, and can this improve risk assessment for allergenic foods?: Report from an ILSI Europe Food Allergy Task Force Expert Group and Workshop. *Allergy*, 73(7): 1383–1392. https://doi.org/10.1111/all.13405

The experts therefore concluded that while cofactors will impact thresholds and severity in some individuals, their impact does not appear to be any greater than the inherent shift in both clinical thresholds and risk of anaphylaxis identified in the wider food-allergic population, nor does it appear that such effects are predictable, as analysed in more detail in Turner *et al.* (2022a). Consumers with food-dependent exercise-induced anaphylaxis (FDEIA) (predominantly to wheat and seafood) may be an exception since such individuals appear to be tolerant to the allergen in the absence of the relevant cofactor. However, the experts noted that for individuals with FDEIA, reaction thresholds are typically 2–3 log greater than the ED_{05} levels under consideration in this report and therefore do not constitute an appropriate basis for deriving conclusions about the consequences of low-dose exposures in individuals with FDEIA. The experts further commented that risk mitigation under such circumstances is probably best managed by health care professionals in providing appropriate advice to consumers at risk of cofactor-dependent reactions.

7.11 SUMMARY AND CONCLUSIONS ON HAZARD CHARACTERIZATION FOR PRIORITY ALLERGENIC FOODS

The experts concluded that at an ED_{05} level of exposure to peanut, around one-third of peanut-allergic participants would experience subjective symptoms, the vast majority of a mild and transient nature. Of the 5 percent of individuals predicted to develop objective symptoms, only 4.5 percent would have anaphylaxis. This equates to 50 peanut-allergic individuals per 1 000 ingesting an ED_{05} exposure dose developing objective symptoms, and 2.3 (of the 50 individuals with objective symptoms) predicted to develop anaphylaxis.

The experts reviewed the analysis by Turner *et al.* (2022a) and agreed that at an ED_{05} level of exposure, there is no evidence to suggest that other priority allergens result in a higher rate of anaphylaxis than peanut (Table 10). Furthermore, Turner *et al.* (2022a) did not identify any cases of anaphylaxis at ≤ED_{05} levels which were refractory to treatment (where administered); indeed, for many of the reports included in their analysis, a significant proportion of anaphylaxis reactions were not treated with epinephrine/adrenaline (reflecting both local variations in interpretation of anaphylaxis criteria and management of reactions by clinicians). At these low levels of exposure, the probability of anaphylaxis would be expected to be ≤0.25 percent. At least 80 percent of these episodes would resolve without treatment, while >97 percent of the remainder would respond to first line treatment (with epinephrine/adrenaline). The risk of fatal reaction to an ED_{05} exposure in an allergic individual is estimated to be <1 per million; to date, there are no reports in the literature of fatal reactions to levels of allergen exposure below 5 mg food protein, for any allergenic food.

Given that the evidence base is strongest for peanut, with data encompassing over 3 000 DBPCFCs reported in the literature (including evidence relating to reproducibility of reaction thresholds and the impact of cofactors) (Turner *et al.*, 2022a), the experts proposes that peanut can be used as an exemplar allergen in terms of hazard characterization.

TABLE 10 **SUMMARY TABLE FOR THE RATE OF ANAPHYLAXIS TO ED05 LEVELS OF EXPOSURE IN ALLERGIC INDIVIDUALS. IT SHOULD BE NOTED THAT THE UPPER LIMIT OF THE 95 PERCENT CI FOR THE CUMULATIVE ED05 FOR SESAME SEED IS NOW ESTIMATED TO BE LESS THAN 58 MG SESAME SEED PROTEIN. AS SUCH, THE RESULTS FOR SESAME SEED IN THIS TABLE ARE CONSIDERED CONSERVATIVE**

ALLERGEN	EVIDENCE BASE (number of FC included in dataset)	UPPER LIMIT OF THE 95% CI FOR CUM ED05 (mg protein)	EXPECTED RATE OF SYMPTOMS TO A LEVEL OF ALLERGEN EXPOSURE ≤ UPPER 95% CI FOR THE CUM ED_{05}		EXPECTED RATE OF ANAPHYLAXIS TO AN ALLERGEN EXPOSURE ≤ UPPER 95% CI FOR THE CUM ED_{05}, AS A PROPORTION OF	
			Any symptoms	Objective symptoms	Individuals reacting to ED_{05} exposure with objective symptoms	All individuals allergic to this food
PEANUT	3 151 DBPCFC	7.1 mg	35–45%	8%	4.5% (95%CI: 1.9% to 10%)	2.3 per 1 000 (95%CI: 1.0 to 5.1 per 1000)
CASHEW	323 DBPCFC 421 Open FC	9.4 mg	32%	12%	4.9% (95%CI: 2.2% to 10.5%)	2.5 per 1 000 (95%CI: 1.1 to 5.3 per 1000)
HAZELNUT	391 DBPCFC 43 Open FC	15.7 mg	~75%	9%	2.5% (95%CI: 0.3% to 15.8%)	1.2 per 1 000 (95%CI: 0.2 to 7.9 per 1000)
WALNUT	194 DBPCFC 156 Open FC	13.0 mg	~60%	14%	5.3% (95%CI: 2.0% to 13%)	2.7 per 1 000 (95%CI: 1.0 to 6.7 per 1000)
SESAME	59 DBPCFC 214 Open FC	58 mg	Not reported	20%	3.0% (95%CI: 0.8% to 11%)	1.5 per 1 000 (95%CI: 0.4 to 5.7 per 1000)
COW'S MILK	728 DBPCFC 317 other FC	6.6 mg	20%	9%	4.9% (95%CI: 2.1% to 11%)	2.5 per 1 000 (95%CI: 1.1 to 5.5 per 1000)
EGG	637 DBPCFC 543 other FC	5.3 mg	14%	9%	1.5% (95%CI: 0.02% to 55%)	0.8 per 1 000 (95%CI: 0 to 27 per 1000)
WHEAT	123 DBPCFC 23 Open FC	25 mg	Not reported	11%	2.2% (95%CI: 0.02% to 75%)	1.1 per 1 000 (95%CI: 0 to 38 per 1000)
FISH	59 DBPCFC	102 mg	58%	25%	Insufficient data for meta-analysis	
SHRIMP	12 DBPCFC 46 Open FC	1 850 mg	57%	19%		
SOYA	89 DBPCFC 51 Open FC	76 mg	Not reported	Not reported	0% (95%CI: 0% to 16.8%)	0 per 1 000 (95%CI: 0 to 8.4 per 1000)

Source: Reproduced with permission from Turner *et al.* (2022a). **Turner, P.J., Patel, N., Ballmer-Weber, B.K., Baumert, J.L., Blom, W.M., Brooke- Taylor, S., Brough, H. *et al.*** 2022a. Peanut can be used as a reference allergen for hazard characterization in food allergen risk management: a rapid evidence assessment and meta-analysis. *The Journal of Allergy and Clinical Immunology: In Practice*, 10(1): 59–70. https:// doi.org/10.1016/j.jaip.2021.08.008

CHAPTER 8

TRANSLATING REFERENCE DOSES (RfD) INTO ACTION LEVELS AND CONSEQUENCES FOR TEST METHOD PERFORMANCE REQUIREMENTS FOR ALLERGEN ANALYSIS

8.1 TRANSLATING REFERENCE DOSES (RFD) TO ACTION LEVELS

As described in Chapter 5, the reference doses are expressed as doses of mg total protein from the allergenic food. To apply these in the context of action levels for PAL and required limits of quantification of analytical methods to monitor compliance of food products with the RfD, these need to be converted into concentrations expressed as mg total protein of the allergenic food per kg food product containing the unintended allergen using the formula:

$$\underset{\text{(in mg total protein from the allergenic food/kg food)}}{\text{AL}} = \frac{\underset{\text{(in mg total protein from the allergenic food)}}{\text{RfD}}}{\underset{\text{(in kg)}}{\text{Amount of food consumed}}}$$

Houben *et al.* (2020) discussed and provided recommendations about the appropriate food intake figures to use for such conversion to avoid under or overestimating the resulting action level, and to produce an accurate risk estimate. Food intake figures representing the use of food items by individuals on single eating occasions (single meals) should be used. Blom *et al.* (2020) further showed that for food allergen risk assessment, such single eating occasion intake data may be derived from food consumption surveys based on the general population, as these were found to not lead to a relevant under or overestimation of the risk for the food-allergic population. Blom *et al.* (2019) in the framework of the European Union iFAAM project previously showed that the 50th percentile value of the population distribution of the single eating occasion intake of foods within a food group resulted in compliance with the safety objective achieved by using the ED_{01} as HBGV in 99 percent of numerous scenarios assessed. Using the 75th percentile extended compliance with that safety objective to 100 percent of the scenarios. Based on these analyses, they suggested that the 75th percentile is the optimal point estimate for use in the deterministic food allergy risk assessment required to meet the safety objective of compliance with the ED_{01} and is adequately conservative for a public health context. When using ED values greater than the ED_{01} as the basis for HBGV, the optimal percentile of the distribution will likely fall within a similar range (in the 50th–75th percentile range) but may need verification by additional sensitivity analyses as conducted by Blom *et al.* (2019). The percentile chosen, appropriate to the risk management objective, is known as the reference amount (RfA).

The subgroup recommends risk assessors and risk managers establish action levels based on the appropriate percentile value (P50 to P75 or adjusted if indicated by additional sensitivity analyses) from the population distribution of the single eating occasion intake of a food. The action level can be calculated from the HBGV expressed as reference dose (RfD) for each allergenic food using the equation above or by using a table based on a list of predefined narrow intake categories (reference amount (RfA) categories of food intake, such as < 10 g, 10 to <20 g, 20 to <30 g, etc. calculated using the upper bound of the interval, see Table 11). For easier reference, the calculated action levels can be rounded down as illustrated in Table 12. The subgroup initially considered intake categories of different increment sizes from 10 g at the lower end (0–10 g) up to 250 g for intakes of 500–750 g. Ultimately, however, they agreed on the use of predefined intake categories with 10 g increment steps as proposed in Table 11 and Table 12. This approach has advantages both at the lower as well as the higher intake ranges. In the lower intake ranges, the increment steps are relatively large, which pushes the relatively high action levels down for food products with intakes below the upper bound of the category. In the higher intake ranges, this effect is negligible and the relatively small incremental steps hardly change the action levels and put less pressure on the analytical sensitivities required.

TABLE 11 **ACTION LEVELS (ALs) FOR PRIORITY ALLERGENS BASED ON RECOMMENDED REFERENCE DOSES (RfDs) AND CALCULATED FOR PREDEFINED INTAKE CATEGORIES (REFERENCE AMOUNTS – RfAs) FROM 10 g TO 1 kg IN INCREMENTAL STEPS OF 10 g. ALs ARE EXPRESSED AS mg TOTAL PROTEIN FROM THE ALLERGENIC FOOD PER kg FOOD**

RfD in mg	CRUSTACEA	FISH	WHEAT	HAZELNUT	SESAME	MILK	EGG	PEANUT	CASHEW	WALNUT
RfA in g	200	5	5	3	2	2	2	2	1	1
10	20 000.00	500.00	500.00	300.00	200.00	200.00	200.00	200.00	100.00	100.00
20	10 000.00	250.00	250.00	150.00	100.00	100.00	100.00	100.00	50.00	50.00
30	6 666.67	166.67	166.67	100.00	66.67	66.67	66.67	66.67	33.33	33.33
40	5 000.00	125.00	125.00	75.00	50.00	50.00	50.00	50.00	25.00	25.00
50	4 000.00	100.00	100.00	60.00	40.00	40.00	40.00	40.00	20.00	20.00
60	3 333.33	83.33	83.33	50.00	33.33	33.33	33.33	33.33	16.67	16.67
70	2 857.14	71.43	71.43	42.86	28.57	28.57	28.57	28.57	14.29	14.29
80	2 500.00	62.50	62.50	37.50	25.00	25.00	25.00	25.00	12.50	12.50
90	2 222.22	55.56	55.56	33.33	22.22	22.22	22.22	22.22	11.11	11.11
100	2 000.00	50.00	50.00	30.00	20.00	20.00	20.00	20.00	10.00	10.00
110	1 818.18	45.45	45.45	27.27	18.18	18.18	18.18	18.18	9.09	9.09
120	1 666.67	41.67	41.67	25.00	16.67	16.67	16.67	16.67	8.33	8.33
130	1 538.46	38.46	38.46	23.08	15.38	15.38	15.38	15.38	7.69	7.69
140	1 428.57	35.71	35.71	21.43	14.29	14.29	14.29	14.29	7.14	7.14
150	1 333.33	33.33	33.33	20.00	13.33	13.33	13.33	13.33	6.67	6.67
160	1 250.00	31.25	31.25	18.75	12.50	12.50	12.50	12.50	6.25	6.25
170	1 176.47	29.41	29.41	17.65	11.76	11.76	11.76	11.76	5.88	5.88
180	1 111.11	27.78	27.78	16.67	11.11	11.11	11.11	11.11	5.56	5.56
190	1 052.63	26.32	26.32	15.79	10.53	10.53	10.53	10.53	5.26	5.26
200	1 000.00	25.00	25.00	15.00	10.00	10.00	10.00	10.00	5.00	5.00
210	952.38	23.81	23.81	14.29	9.52	9.52	9.52	9.52	4.76	4.76
220	909.09	22.73	22.73	13.64	9.09	9.09	9.09	9.09	4.55	4.55
230	869.57	21.74	21.74	13.04	8.70	8.70	8.70	8.70	4.35	4.35
240	833.33	20.83	20.83	12.50	8.33	8.33	8.33	8.33	4.17	4.17
250	800.00	20.00	20.00	12.00	8.00	8.00	8.00	8.00	4.00	4.00
260	769.23	19.23	19.23	11.54	7.69	7.69	7.69	7.69	3.85	3.85
270	740.74	18.52	18.52	11.11	7.41	7.41	7.41	7.41	3.70	3.70
280	714.29	17.86	17.86	10.71	7.14	7.14	7.14	7.14	3.57	3.57
290	689.66	17.24	17.24	10.34	6.90	6.90	6.90	6.90	3.45	3.45
300	666.67	16.67	16.67	10.00	6.67	6.67	6.67	6.67	3.33	3.33
310	645.16	16.13	16.13	9.68	6.45	6.45	6.45	6.45	3.23	3.23
320	625.00	15.63	15.63	9.38	6.25	6.25	6.25	6.25	3.13	3.13
330	606.06	15.15	15.15	9.09	6.06	6.06	6.06	6.06	3.03	3.03
340	588.24	14.71	14.71	8.82	5.88	5.88	5.88	5.88	2.94	2.94
350	571.43	14.29	14.29	8.57	5.71	5.71	5.71	5.71	2.86	2.86
360	555.56	13.89	13.89	8.33	5.56	5.56	5.56	5.56	2.78	2.78
370	540.54	13.51	13.51	8.11	5.41	5.41	5.41	5.41	2.70	2.70
380	526.32	13.16	13.16	7.89	5.26	5.26	5.26	5.26	2.63	2.63
390	512.82	12.82	12.82	7.69	5.13	5.13	5.13	5.13	2.56	2.56
400	500.00	12.50	12.50	7.50	5.00	5.00	5.00	5.00	2.50	2.50

TABLE 11 **ACTION LEVELS (ALs) FOR PRIORITY ALLERGENS BASED ON RECOMMENDED REFERENCE DOSES (RfDs) AND CALCULATED FOR PREDEFINED INTAKE CATEGORIES (REFERENCE AMOUNTS – RfAs) FROM 10 g TO 1 kg IN INCREMENTAL STEPS OF 10 g. ALs ARE EXPRESSED AS mg TOTAL PROTEIN FROM THE ALLERGENIC FOOD PER kg FOOD** (continued)

RfD in mg	CRUSTACEA	FISH	WHEAT	HAZELNUT	SESAME	MILK	EGG	PEANUT	CASHEW	WALNUT
RfA in g	200	5	5	3	2	2	2	2	1	1
410	487.80	12.20	12.20	7.32	4.88	4.88	4.88	4.88	2.44	2.44
420	476.19	11.90	11.90	7.14	4.76	4.76	4.76	4.76	2.38	2.38
430	465.12	11.63	11.63	6.98	4.65	4.65	4.65	4.65	2.33	2.33
440	454.55	11.36	11.36	6.82	4.55	4.55	4.55	4.55	2.27	2.27
450	444.44	11.11	11.11	6.67	4.44	4.44	4.44	4.44	2.22	2.22
460	434.78	10.87	10.87	6.52	4.35	4.35	4.35	4.35	2.17	2.17
470	425.53	10.64	10.64	6.38	4.26	4.26	4.26	4.26	2.13	2.13
480	416.67	10.42	10.42	6.25	4.17	4.17	4.17	4.17	2.08	2.08
490	408.16	10.20	10.20	6.12	4.08	4.08	4.08	4.08	2.04	2.04
500	400.00	10.00	10.00	6.00	4.00	4.00	4.00	4.00	2.00	2.00
510	392.16	9.80	9.80	5.88	3.92	3.92	3.92	3.92	1.96	1.96
520	384.62	9.62	9.62	5.77	3.85	3.85	3.85	3.85	1.92	1.92
530	377.36	9.43	9.43	5.66	3.77	3.77	3.77	3.77	1.89	1.89
540	370.37	9.26	9.26	5.56	3.70	3.70	3.70	3.70	1.85	1.85
550	363.64	9.09	9.09	5.45	3.64	3.64	3.64	3.64	1.82	1.82
560	357.14	8.93	8.93	5.36	3.57	3.57	3.57	3.57	1.79	1.79
570	350.88	8.77	8.77	5.26	3.51	3.51	3.51	3.51	1.75	1.75
580	344.83	8.62	8.62	5.17	3.45	3.45	3.45	3.45	1.72	1.72
590	338.98	8.47	8.47	5.08	3.39	3.39	3.39	3.39	1.69	1.69
600	333.33	8.33	8.33	5.00	3.33	3.33	3.33	3.33	1.67	1.67
610	327.87	8.20	8.20	4.92	3.28	3.28	3.28	3.28	1.64	1.64
620	322.58	8.06	8.06	4.84	3.23	3.23	3.23	3.23	1.61	1.61
630	317.46	7.94	7.94	4.76	3.17	3.17	3.17	3.17	1.59	1.59
640	312.50	7.81	7.81	4.69	3.13	3.13	3.13	3.13	1.56	1.56
650	307.69	7.69	7.69	4.62	3.08	3.08	3.08	3.08	1.54	1.54
660	303.03	7.58	7.58	4.55	3.03	3.03	3.03	3.03	1.52	1.52
670	298.51	7.46	7.46	4.48	2.99	2.99	2.99	2.99	1.49	1.49
680	294.12	7.35	7.35	4.41	2.94	2.94	2.94	2.94	1.47	1.47
690	289.86	7.25	7.25	4.35	2.90	2.90	2.90	2.90	1.45	1.45
700	285.71	7.14	7.14	4.29	2.86	2.86	2.86	2.86	1.43	1.43
710	281.69	7.04	7.04	4.23	2.82	2.82	2.82	2.82	1.41	1.41
720	277.78	6.94	6.94	4.17	2.78	2.78	2.78	2.78	1.39	1.39
730	273.97	6.85	6.85	4.11	2.74	2.74	2.74	2.74	1.37	1.37
740	270.27	6.76	6.76	4.05	2.70	2.70	2.70	2.70	1.35	1.35
750	266.67	6.67	6.67	4.00	2.67	2.67	2.67	2.67	1.33	1.33
760	263.16	6.58	6.58	3.95	2.63	2.63	2.63	2.63	1.32	1.32
770	259.74	6.49	6.49	3.90	2.60	2.60	2.60	2.60	1.30	1.30
780	256.41	6.41	6.41	3.85	2.56	2.56	2.56	2.56	1.28	1.28
790	253.16	6.33	6.33	3.80	2.53	2.53	2.53	2.53	1.27	1.27
800	250.00	6.25	6.25	3.75	2.50	2.50	2.50	2.50	1.25	1.25

TABLE 11 **ACTION LEVELS (ALs) FOR PRIORITY ALLERGENS BASED ON RECOMMENDED REFERENCE DOSES (RfDs) AND CALCULATED FOR PREDEFINED INTAKE CATEGORIES (REFERENCE AMOUNTS – RfAs) FROM 10 g TO 1 kg IN INCREMENTAL STEPS OF 10 g. ALs ARE EXPRESSED AS mg TOTAL PROTEIN FROM THE ALLERGENIC FOOD PER kg FOOD** (continued)

RfD in mg	CRUSTACEA	FISH	WHEAT	HAZELNUT	SESAME	MILK	EGG	PEANUT	CASHEW	WALNUT
RfA in g	200	5	5	3	2	2	2	2	1	1
810	246.91	6.17	6.17	3.70	2.47	2.47	2.47	2.47	1.23	1.23
820	243.90	6.10	6.10	3.66	2.44	2.44	2.44	2.44	1.22	1.22
830	240.96	6.02	6.02	3.61	2.41	2.41	2.41	2.41	1.20	1.20
840	238.10	5.95	5.95	3.57	2.38	2.38	2.38	2.38	1.19	1.19
850	235.29	5.88	5.88	3.53	2.35	2.35	2.35	2.35	1.18	1.18
860	232.56	5.81	5.81	3.49	2.33	2.33	2.33	2.33	1.16	1.16
870	229.89	5.75	5.75	3.45	2.30	2.30	2.30	2.30	1.15	1.15
880	227.27	5.68	5.68	3.41	2.27	2.27	2.27	2.27	1.14	1.14
890	224.72	5.62	5.62	3.37	2.25	2.25	2.25	2.25	1.12	1.12
900	222.22	5.56	5.56	3.33	2.22	2.22	2.22	2.22	1.11	1.11
910	219.78	5.49	5.49	3.30	2.20	2.20	2.20	2.20	1.10	1.10
920	217.39	5.43	5.43	3.26	2.17	2.17	2.17	2.17	1.09	1.09
930	215.05	5.38	5.38	3.23	2.15	2.15	2.15	2.15	1.08	1.08
940	212.77	5.32	5.32	3.19	2.13	2.13	2.13	2.13	1.06	1.06
950	210.53	5.26	5.26	3.16	2.11	2.11	2.11	2.11	1.05	1.05
960	208.33	5.21	5.21	3.13	2.08	2.08	2.08	2.08	1.04	1.04
970	206.19	5.15	5.15	3.09	2.06	2.06	2.06	2.06	1.03	1.03
980	204.08	5.10	5.10	3.06	2.04	2.04	2.04	2.04	1.02	1.02
990	202.02	5.05	5.05	3.03	2.02	2.02	2.02	2.02	1.01	1.01
1000	200.00	5.00	5.00	3.00	2.00	2.00	2.00	2.00	1.00	1.00

Source: Authors' own elaboration.

TABLE 12 **ACTION LEVELS (ALs) FROM TABLE 11 BUT ROUNDED DOWN FOR CLARITY AND EASE OF USE. ALs ARE EXPRESSED AS mg TOTAL PROTEIN FROM THE ALLERGENIC FOOD PER kg FOOD**

RfD in mg	CRUSTACEA	FISH	WHEAT	HAZELNUT	SESAME	MILK	EGG	PEANUT	CASHEW	WALNUT
RFA in g	200	5	5	3	2	2	2	2	1	1
10	20 000	500	500	300	200	200	200	200	100	100
20	10 000	250	250	150	100	100	100	100	50	50
30	6 500	150	150	100	65	65	65	65	30	30
40	5 000	100	100	75	50	50	50	50	25	25
50	4 000	100	100	60	40	40	40	40	20	20
60	3 000	80	80	50	30	30	30	30	15	15
70	2 500	70	70	40	25	25	25	25	10	10
80	2 500	60	60	35	25	25	25	25	10	10
90	2 000	55	55	30	20	20	20	20	10	10
100	2 000	50	50	30	20	20	20	20	10	10
110	1 500	45	45	25	15	15	15	15	9	9
120	1 500	40	40	25	15	15	15	15	8	8
130	1 500	35	35	20	15	15	15	15	7	7
140	1 000	35	35	20	10	10	10	10	7	7
150	1 000	30	30	20	10	10	10	10	6	6
160	1 000	30	30	15	10	10	10	10	6	6
170	1 000	25	25	15	10	10	10	10	5	5
180	1 000	25	25	15	10	10	10	10	5	5
190	1 000	25	25	15	10	10	10	10	5	5
200	1 000	25	25	15	10	10	10	10	5	5
210	950	20	20	10	9	9	9	9	4	4
220	900	20	20	10	9	9	9	9	4	4
230	850	20	20	10	8	8	8	8	4	4
240	800	20	20	10	8	8	8	8	4	4
250	800	20	20	10	8	8	8	8	4	4
260	750	15	15	10	7	7	7	7	3	3
270	700	15	15	10	7	7	7	7	3	3
280	700	15	15	10	7	7	7	7	3	3
290	650	15	15	10	6	6	6	6	3	3
300	650	15	15	10	6	6	6	6	3	3
310	600	15	15	9	6	6	6	6	3	3
320	600	15	15	9	6	6	6	6	3	3
330	600	15	15	9	6	6	6	6	3	3
340	550	10	10	8	5	5	5	5	2	2
350	550	10	10	8	5	5	5	5	2	2
360	550	10	10	8	5	5	5	5	2	2
370	500	10	10	8	5	5	5	5	2	2
380	500	10	10	7	5	5	5	5	2	2
390	500	10	10	7	5	5	5	5	2	2
400	500	10	10	7	5	5	5	5	2	2

TABLE 12 **ACTION LEVELS (ALs) FROM TABLE 11 BUT ROUNDED DOWN FOR CLARITY AND EASE OF USE. ALs ARE EXPRESSED AS mg TOTAL PROTEIN FROM THE ALLERGENIC FOOD PER kg FOOD** (continued)

RfD in mg	CRUSTACEA	FISH	WHEAT	HAZELNUT	SESAME	MILK	EGG	PEANUT	CASHEW	WALNUT
RFA in g	200	5	5	3	2	2	2	2	1	1
410	450	10	10	7	4	4	4	4	2	2
420	450	10	10	7	4	4	4	4	2	2
430	450	10	10	6	4	4	4	4	2	2
440	450	10	10	6	4	4	4	4	2	2
450	400	10	10	6	4	4	4	4	2	2
460	400	10	10	6	4	4	4	4	2	2
470	400	10	10	6	4	4	4	4	2	2
480	400	10	10	6	4	4	4	4	2	2
490	400	10	10	6	4	4	4	4	2	2
500	400	10	10	6	4	4	4	4	2	2
510	350	9	9	5	3	3	3	3	1	1
520	350	9	9	5	3	3	3	3	1	1
530	350	9	9	5	3	3	3	3	1	1
540	350	9	9	5	3	3	3	3	1	1
550	350	9	9	5	3	3	3	3	1	1
560	350	8	8	5	3	3	3	3	1	1
570	350	8	8	5	3	3	3	3	1	1
580	300	8	8	5	3	3	3	3	1	1
590	300	8	8	5	3	3	3	3	1	1
600	300	8	8	5	3	3	3	3	1	1
610	300	8	8	4	3	3	3	3	1	1
620	300	8	8	4	3	3	3	3	1	1
630	300	7	7	4	3	3	3	3	1	1
640	300	7	7	4	3	3	3	3	1	1
650	300	7	7	4	3	3	3	3	1	1
660	300	7	7	4	3	3	3	3	1	1
670	250	7	7	4	2	2	2	2	1	1
680	250	7	7	4	2	2	2	2	1	1
690	250	7	7	4	2	2	2	2	1	1
700	250	7	7	4	2	2	2	2	1	1
710	250	7	7	4	2	2	2	2	1	1
720	250	6	6	4	2	2	2	2	1	1
730	250	6	6	4	2	2	2	2	1	1
740	250	6	6	4	2	2	2	2	1	1
750	250	6	6	4	2	2	2	2	1	1
760	250	6	6	3	2	2	2	2	1	1
770	250	6	6	3	2	2	2	2	1	1
780	250	6	6	3	2	2	2	2	1	1
790	250	6	6	3	2	2	2	2	1	1
800	250	6	6	3	2	2	2	2	1	1

TABLE 12 **ACTION LEVELS (ALs) FROM TABLE 11 BUT ROUNDED DOWN FOR CLARITY AND EASE OF USE. ALs ARE EXPRESSED AS mg TOTAL PROTEIN FROM THE ALLERGENIC FOOD PER kg FOOD** (continued)

RfD in mg	CRUSTACEA	FISH	WHEAT	HAZELNUT	SESAME	MILK	EGG	PEANUT	CASHEW	WALNUT
RFA in g	200	5	5	3	2	2	2	2	1	1
810	200	6	6	3	2	2	2	2	1	1
820	200	6	6	3	2	2	2	2	1	1
830	200	6	6	3	2	2	2	2	1	1
840	200	5	5	3	2	2	2	2	1	1
850	200	5	5	3	2	2	2	2	1	1
860	200	5	5	3	2	2	2	2	1	1
870	200	5	5	3	2	2	2	2	1	1
880	200	5	5	3	2	2	2	2	1	1
890	200	5	5	3	2	2	2	2	1	1
900	200	5	5	3	2	2	2	2	1	1
910	200	5	5	3	2	2	2	2	1	1
920	200	5	5	3	2	2	2	2	1	1
930	200	5	5	3	2	2	2	2	1	1
940	200	5	5	3	2	2	2	2	1	1
950	200	5	5	3	2	2	2	2	1	1
960	200	5	5	3	2	2	2	2	1	1
970	200	5	5	3	2	2	2	2	1	1
980	200	5	5	3	2	2	2	2	1	1
990	200	5	5	3	2	2	2	2	1	1
1000	200	5	5	3	2	2	2	2	1	1

Source: Authors'own elaboration.

Method performance criteria indicate that the limits of quantification (LoQ) of any method utilized for a specific food should be around 3-fold lower than the action level for that food in order to account for real-world performance variability and to assure that the analytical result is truly at or below the action level.

TABLE 13 **LIMITS OF QUANTIFICATION (LoQ) REQUIRED FOR ANALYTICAL METHODS TO MEET CALCULATED ALs (TABLES 11 AND 12), TAKING INTO ACCOUNT METHOD PERFORMANCE**

RfD in mg	CRUSTACEA	FISH	WHEAT	HAZELNUT	SESAME	MILK	EGG	PEANUT	CASHEW	WALNUT
RFA in g	200	5	5	3	2	2	2	2	1	1
10	6 666	166	166	100	66	66	66	66	33	33
20	3 333	83	83	50	33	33	33	33	16	16
30	2 166	50	50	33	21	21	21	21	10	10
40	1 666	33	33	25	16	16	16	16	8.3	8.3
50	1 333	33	33	20	13	13	13	13	6.6	6.6
60	1 000	26	26	16	10	10	10	10	5	5
70	833	23	23	13	8.3	8.3	8.3	8.3	3.3	3.3
80	833	20	20	11	8.3	8.3	8.3	8.3	3.3	3.3
90	666	18	18	10	6.6	6.6	6.6	6.6	3.3	3.3
100	666	16	16	10	6.6	6.6	6.6	6.6	3.3	3.3
110	500	15	15	8.3	5	5	5	5	3	3
120	500	13	13	8.3	5	5	5	5	2.6	2.6
130	500	11	11	6.6	5	5	5	5	2.3	2.3
140	333	11	11	6.6	3.3	3.3	3.3	3.3	2.3	2.3
150	333	10	10	6.6	3.3	3.3	3.3	3.3	2	2
160	333	10	10	5	3.3	3.3	3.3	3.3	2	2
170	333	8.3	8.3	5	3.3	3.3	3.3	3.3	1.6	1.6
180	333	8.3	8.3	5	3.3	3.3	3.3	3.3	1.6	1.6
190	333	8.3	8.3	5	3.3	3.3	3.3	3.3	1.6	1.6
200	333	8.3	8.3	5	3.3	3.3	3.3	3.3	1.6	1.6
210	316	6.6	6.6	3.3	3	3	3	3	1.3	1.3
220	300	6.6	6.6	3.3	3	3	3	3	1.3	1.3
230	283	6.6	6.6	3.3	2.6	2.6	2.6	2.6	1.3	1.3
240	266	6.6	6.6	3.3	2.6	2.6	2.6	2.6	1.3	1.3
250	266	6.6	6.6	3.3	2.6	2.6	2.6	2.6	1.3	1.3
260	250	5	5	3.3	2.3	2.3	2.3	2.3	1	1
270	233	5	5	3.3	2.3	2.3	2.3	2.3	1	1
280	233	5	5	3.3	2.3	2.3	2.3	2.3	1	1
290	216	5	5	3.3	2	2	2	2	1	1
300	216	5	5	3.3	2	2	2	2	1	1
310	200	5	5	3	2	2	2	2	1	1
320	200	5	5	3	2	2	2	2	1	1
330	200	5	5	3	2	2	2	2	1	1
340	183	3.3	3.3	2.6	1.6	1.6	1.6	1.6	0.6	0.6
350	183	3.3	3.3	2.6	1.6	1.6	1.6	1.6	0.6	0.6

TABLE 13 **LIMITS OF QUANTIFICATION (LoQ) REQUIRED FOR ANALYTICAL METHODS TO MEET CALCULATED ALs (TABLES 11 AND 12), TAKING INTO ACCOUNT METHOD PERFORMANCE** (continued)

RfD in mg	CRUSTACEA	FISH	WHEAT	HAZELNUT	SESAME	MILK	EGG	PEANUT	CASHEW	WALNUT
RFA in g	200	5	5	3	2	2	2	2	1	1
360	183	3.3	3.3	2.6	1.6	1.6	1.6	1.6	0.6	0.6
370	166	3.3	3.3	2.6	1.6	1.6	1.6	1.6	0.6	0.6
380	166	3.3	3.3	2.3	1.6	1.6	1.6	1.6	0.6	0.6
390	166	3.3	3.3	2.3	1.6	1.6	1.6	1.6	0.6	0.6
400	166	3.3	3.3	2.3	1.6	1.6	1.6	1.6	0.6	0.6
410	150	3.3	3.3	2.3	1.3	1.3	1.3	1.3	0.6	0.6
420	150	3.3	3.3	2.3	1.3	1.3	1.3	1.3	0.6	0.6
430	150	3.3	3.3	2	1.3	1.3	1.3	1.3	0.6	0.6
440	150	3.3	3.3	2	1.3	1.3	1.3	1.3	0.6	0.6
450	133	3.3	3.3	2	1.3	1.3	1.3	1.3	0.6	0.6
460	133	3.3	3.3	2	1.3	1.3	1.3	1.3	0.6	0.6
470	133	3.3	3.3	2	1.3	1.3	1.3	1.3	0.6	0.6
480	133	3.3	3.3	2	1.3	1.3	1.3	1.3	0.6	0.6
490	133	3.3	3.3	2	1.3	1.3	1.3	1.3	0.6	0.6
500	133	3.3	3.3	2	1.3	1.3	1.3	1.3	0.6	0.6
510	116	3	3	1.6	1	1	1	1	0.3	0.3
520	116	3	3	1.6	1	1	1	1	0.3	0.3
530	116	3	3	1.6	1	1	1	1	0.3	0.3
540	116	3	3	1.6	1	1	1	1	0.3	0.3
550	116	3	3	1.6	1	1	1	1	0.3	0.3
560	116	2.6	2.6	1.6	1	1	1	1	0.3	0.3
570	116	2.6	2.6	1.6	1	1	1	1	0.3	0.3
580	100	2.6	2.6	1.6	1	1	1	1	0.3	0.3
590	100	2.6	2.6	1.6	1	1	1	1	0.3	0.3
600	100	2.6	2.6	1.6	1	1	1	1	0.3	0.3
610	100	2.6	2.6	1.3	1	1	1	1	0.3	0.3
620	100	2.6	2.6	1.3	1	1	1	1	0.3	0.3
630	100	2.3	2.3	1.3	1	1	1	1	0.3	0.3
640	100	2.3	2.3	1.3	1	1	1	1	0.3	0.3
650	100	2.3	2.3	1.3	1	1	1	1	0.3	0.3
660	100	2.3	2.3	1.3	1	1	1	1	0.3	0.3
670	83	2.3	2.3	1.3	0.6	0.6	0.6	0.6	0.3	0.3
680	83	2.3	2.3	1.3	0.6	0.6	0.6	0.6	0.3	0.3
690	83	2.3	2.3	1.3	0.6	0.6	0.6	0.6	0.3	0.3
700	83	2.3	2.3	1.3	0.6	0.6	0.6	0.6	0.3	0.3
710	83	2.3	2.3	1.3	0.6	0.6	0.6	0.6	0.3	0.3
720	83	2	2	1.3	0.6	0.6	0.6	0.6	0.3	0.3
730	83	2	2	1.3	0.6	0.6	0.6	0.6	0.3	0.3
740	83	2	2	1.3	0.6	0.6	0.6	0.6	0.3	0.3
750	83	2	2	1.3	0.6	0.6	0.6	0.6	0.3	0.3
760	83	2	2	1	0.6	0.6	0.6	0.6	0.3	0.3

TABLE 13 **LIMITS OF QUANTIFICATION (LoQ) REQUIRED FOR ANALYTICAL METHODS TO MEET CALCULATED ALs (TABLES 11 AND 12), TAKING INTO ACCOUNT METHOD PERFORMANCE** (continued)

RfD in mg	CRUSTACEA	FISH	WHEAT	HAZELNUT	SESAME	MILK	EGG	PEANUT	CASHEW	WALNUT
RFA in g	200	5	5	3	2	2	2	2	1	1
770	83	2	2	1	0.6	0.6	0.6	0.6	0.3	0.3
780	83	2	2	1	0.6	0.6	0.6	0.6	0.3	0.3
790	83	2	2	1	0.6	0.6	0.6	0.6	0.3	0.3
800	83	2	2	1	0.6	0.6	0.6	0.6	0.3	0.3
810	66	2	2	1	0.6	0.6	0.6	0.6	0.3	0.3
820	66	2	2	1	0.6	0.6	0.6	0.6	0.3	0.3
830	66	2	2	1	0.6	0.6	0.6	0.6	0.3	0.3
840	66	1.6	1.6	1	0.6	0.6	0.6	0.6	0.3	0.3
850	66	1.6	1.6	1	0.6	0.6	0.6	0.6	0.3	0.3
860	66	1.6	1.6	1	0.6	0.6	0.6	0.6	0.3	0.3
870	66	1.6	1.6	1	0.6	0.6	0.6	0.6	0.3	0.3
880	66	1.6	1.6	1	0.6	0.6	0.6	0.6	0.3	0.3
890	66	1.6	1.6	1	0.6	0.6	0.6	0.6	0.3	0.3
900	66	1.6	1.6	1	0.6	0.6	0.6	0.6	0.3	0.3
910	66	1.6	1.6	1	0.6	0.6	0.6	0.6	0.3	0.3
920	66	1.6	1.6	1	0.6	0.6	0.6	0.6	0.3	0.3
930	66	1.6	1.6	1	0.6	0.6	0.6	0.6	0.3	0.3
940	66	1.6	1.6	1	0.6	0.6	0.6	0.6	0.3	0.3
950	66	1.6	1.6	1	0.6	0.6	0.6	0.6	0.3	0.3
960	66	1.6	1.6	1	0.6	0.6	0.6	0.6	0.3	0.3
970	66	1.6	1.6	1	0.6	0.6	0.6	0.6	0.3	0.3
980	66	1.6	1.6	1	0.6	0.6	0.6	0.6	0.3	0.3
990	66	1.6	1.6	1	0.6	0.6	0.6	0.6	0.3	0.3
1000	66	1.6	1.6	1	0.6	0.6	0.6	0.6	0.3	0.3

Source: Authors'own elaboration.

TABLE 14 **EXAMPLES OF FOOD CONSUMPTION P75 SUMMARY STATISTICS PER FOOD GROUP**

iFAAM GROUP	iFAAM NAME	COUNTRY	P75 (GRAMS)
14	Supplements	Combined	2
39	Spices and salt	Combined	3
23	Chewing gum	Netherlands	4
5	Coffee creamer	Combined	6
23	Chewing gum	France	6
23	Chewing gum	Denmark	10
47	Binding agent	Combined	13
26	Vegetable oils and animal fat	Combined	20
27	Butter/halvarine/margarine	Combined	20
38	Herbs and spices mixes, bouillon cubes, yeast extract	Denmark	20
19	Sugar	Denmark	24
4	Milk powder and cocoa powder	Combined	26
18	Small sweets – sweet confectionary specified	Combined	28
35	Crackers, crisp bread, rusk and toast	Combined	28
6	Cream and coffee milk	Combined	30
28	Sauces used as condiments and dessert sauces	Combined	30
22	Cereal bars	Combined	32
43	Syrups	Combined	34
2	Peanut butter	Combined	35
21	Sweet confectionary (jam, marmalade)	Combined	35
10	Peanuts, nuts and dried fruits	Combined	40
20	Chocolate and chocolate products	Combined	40
31	Fish products – 75 g	Combined	40
45	Cookies (biscuits)	Combined	42
3	Cheese	Combined	48
53	Eggs	Combined	55
11	Potato and other starch based chips (including salty sticks)	Combined	59
17	Small sweets – sweet confectionary unspecified/combined	Combined	60
48	Breakfast products eaten unprocessed (e.g. müsli, oat and maize flakes)	Combined	60
29	Sauces, savory, chutneys and pickles	Combined	75
33	Meat products - mean 65 g	France	75
41	Alcoholic drinks, alcohol above 15%	Combined	84
12	Fried/warm snacks	Netherlands	86
1	Chestnut paste and coconut milk	Combined	100
7	Ice cream	Combined	100
15	Pancakes and waffles	Netherlands	100
30	Fish products – mean 35 g	Combined	100
13	Meal replacements and meat imitations	Combined	113
36	Bread, bread rolls and bread doughs	Combined	120
34	Meat products – mean 105 g	Combined	126
12	Fried/warm snacks	France	140
32	Fish products – mean 115 g	Combined	150

TABLE 14 **EXAMPLES OF FOOD CONSUMPTION P75 SUMMARY STATISTICS PER FOOD GROUP** (continued)

iFAAM GROUP	iFAAM NAME	COUNTRY	P75 (GRAMS)
51	Legumes	Combined	175
12	Fried/warm snacks	Denmark	180
46	Cakes (including pastry)	Combined	180
54	Egg-based dishes	Combined	180
52	Fruit and vegetables, processed	Combined	190
9	Milk and milk products consumed with a spoon	Combined	200
15	Pancakes and waffles	Denmark	200
15	Pancakes and waffles	France	200
24	Mashed potato powder	Combined	200
50	Pasta, rice, couscous and other grains	Combined	200
49	Breakfast products, porridge	Denmark	202
25	Potato product (excl. powder)	Combined	225
40	Alcoholic drinks, alcohol ≤15%	Combined	283
8	Milk and milk products for drinking	Combined	318
56	Composite dishes	Combined	320
55	Sandwich and pizza	Combined	335
16	Soups	Combined	400
44	Drinks without alcohol (excl. syrup)	Combined	483
42	Beer	Combined	660

Source: Authors' own elaboration.

The values originate from a combined analysis using data from three European countries: the Netherlands, France and Denmark. The analyses resulted in merged consumption data for a number of food groups from the three countries based on defined criteria. (Table derived from values reported in Birot *et al.*, 2018). Note: This example is for illustrative purposes only and is not meant for use by global risk assessors or risk managers.

8.2 TEST METHOD PERFORMANCE REQUIREMENTS – GENERAL CONSIDERATIONS IN LIGHT OF REFERENCE DOSES (RfDs) AND ACTION LEVELS FOR ALLERGENS IN FOODS

General considerations for allergen analysis in food: Since it is preferable to use analytical methods that quantify the hazardous constituent on which the RfDs are based – which in the case of allergenic food ingredients is almost without exception the protein component – this narrative focuses on the two main methods used to directly measure the protein components of allergenic foods. These are enzyme-linked immunosorbent assays (ELISA) and mass spectrometry (MS) methods, with an emphasis on the former because of its wider use and consequently the larger underlying evidence base. Although it is preferable for allergen test methods to target protein, in some instances where such test methodology is lacking, alternative methods, such as those based on DNA, may need to be used.

There can be large variation in test method results obtained on repeat analysis of the same samples by the same method. Food processing and the matrix are some of the factors that affect analytical performance and contribute to test result variability, but many other methodological and sampling issues also do the same. These have been considered in turn below.

Assay sensitivity: Many standardization organizations have excellent documents on determination of assay sensitivity parameters in relation to food contaminants which are relevant for allergen analysis and on which the approaches described here build for allergen analysis by ELISA and MS. These include the IUPAC recommendations (Thompson *et al.*, 2002) on which many other approaches have been based including the AOAC Standard Method Performance Requirement (SMPR) Documents and recommendations of the European Union Joint Research Centre (Wenzel *et al.*, 2016) and Eurachem (Magnusson and Örnemark, 2014).

> ***Limit of Detection (LoD):*** The LoD refers to the lowest level or concentration of analyte that can be differentiated from a sample blank (i.e. extract of a food sample that does not contain the allergen) at a specified probability level. This parameter should only be used as a method performance characteristic, and it is not suitable for reporting on concentrations of unintended allergen presence. IUPAC recommends the precision estimate used to calculate the LoD be based on at least six independent complete determinations of analyte concentration in a typical matrix blank or low-level material, with no censoring of zero or negative results, and the approximate detection limit calculated as three times the precision estimate used (e.g. standard deviation) (Thompson *et al.*, 2002).

> ***Limit of Quantitation (LoQ):*** The LoQ refers to the lowest concentration of analyte in a test sample that can be reasonably quantified at a specified level of precision. IUPAC recommends that "the uncertainty of measurement *[should be expressed]* as a function of concentration and compare that function with a criterion of fitness for purpose agreed between the laboratory and the client or end-user of the data" (Thompson *et al.*, 2002). For allergen analysis, based on experience with method performance, it is recommended that test methods should be able to report quantitative test results 3-fold below the action level. Given the variability in test methods, it is also recommended that analytical laboratories should routinely monitor the LoQ of a given test method. Ideally, the standard curve prepared with the tested sample should include a concentration at the LoQ.

Assay specificity: Manufacturers of commercial ELISA kits frequently fail to provide information on the proteins that the kits recognize, and there is a lack of published data on these aspects. This is in contrast to MS methods where peptide targets are clearly defined. Assay cross-reactivity can be an issue since it can affect assay specificity. It may be that the cross-reactivity of a food has not been comprehensively checked by a kit manufacturer to ensure that a negative is a negative or that a positive is a true positive. Assay specificity can also be important to inform the choice of assay used (e.g. whey vs casein when analysing for milk components).

In some instances, assays maybe be specific for limited types of a wider class of foods, notably when the designation covers a whole subphylum (e.g. crustacea) or refers to a paraphyletic group (e.g. fish). For example, there are ELISAs for tropomyosin, but these have not been validated for cross-reactivity with all the different crustacean foods. Some examples of these issues are given below.

Choice of analytical targets: Allergenic food ingredients comprise complex mixtures of proteins which may exhibit post-translational modifications. Many types of protein species (termed allergen molecules here) that bind allergic patient serum IgE have been described and can be classified based on the protein families to which they belong (Jenkins *et al.*, 2005, 2007), reflecting their evolutionary biology (Radauer and Breiteneder, 2007). However, the relationship between IgE binding and the ability of an allergen to trigger a clinically relevant IgE-mediated reaction is often unclear, as molecular diagnostics have shown (Matricardi *et al.*, 2016). Consequently, major clinically relevant allergen molecules have only been identified for a few foods, such as peanut, where sensitization to the allergen Ara h 2 is of high clinical relevance along with other allergens such as Ara h 1, Ara h 3 and Ara h 6 (Nicolaou *et al.*, 2011; Hemmings *et al.*, 2020). Many of the major allergens are also major components of allergenic ingredients since they include seed storage proteins in plant-derived foods (such as the 7S and 11S seed storage globulins and the 2S albumins) as well as caseins in milk and ovomucoid and ovalbumin in egg. Thus, using such components as analytical targets can help ensure test methods quantify relevant protein components which are abundant and can help support effective test method sensitivity, ensuring test method results relate to RfDs (as RfDs are formulated as a mass of the total protein from the allergenic source). However, the precise targets recognized by ELISA methods are not generally well described, nor is the specification of the material which has been used to raise the antibody preparations, although it is evident for those limited studies which have been undertaken that commercial test kits vary in the allergen molecules they recognize, as has been established for peanut (Jayasena *et al.*, 2015) and milk (Ivens, Baumert and Taylor, 2016). Greater transparency on the precise allergen targets recognized by ELISA methods is essential to inform the choice of the correct test methods. This is less problematic for mass spectrometry methods where peptide targets are clearly described.

Specialist food ingredients which are increasingly available and which may represent highly purified fractions of the original food – as exemplified by specialist cow's milk ingredients such as α-lactalbumin isolates and lactoferrin as well as processing aids such as hen egg lysozyme – may present problems for detection by certain test methods, as do foods in which an ingredient is formulated such that protein fractions are in a ratio different from the ratio in the original ingredient. It is unclear what the allergenic risk posed by such ingredients is in terms of RfD based on whole ingredient protein levels, but care must be taken in selecting analytical methods capable of quantifying such specialist food ingredients. Transparency over analytical targets in test methods would aid in this regard.

Test methods also need to take into account the fact that allergens maybe subject to post-translational processing, including glycosylation, phosphorylation and formation of hydroxyproline amongst others. For food allergens this is compounded by processing-induced modifications, notably Maillard modifications and effects of hydrolytic treatments which may induce deamidation, both of which are thought to modify allergenicity (Toda *et al.*, 2019; Pilolli *et al.*, 2021; Denery-Papini *et al.*, 2012; Parker *et al.*, 2015; Sayers *et al.*, 2016; Khuda *et al.*, 2012a, 2012b). Such post-translational and processing induced modifications may impact both antibody reactivity and utility of peptide targets used in MS methods either as a consequence of loss of tryptic cleavage sites reducing peptide yields or modification of the target peptide *per se* (Pilolli *et al.*, 2020). This is also pertinent to detection of hydrolysed allergenic food ingredients. Specifically, antibodies raised to intact proteins detect peptides poorly or not at all, or it may no longer be possible to generate peptide targets by the proteolytic sample preparation workflows used in MS methods. This has been observed particularly in relation to detection of gluten in soy sauce where there is also an issue that soy sauce may not elicit reactions in allergic or intolerant subjects due to extensive protein modification during the fermentation process (Cao *et al.*, 2017). Hydrolysis is also used to develop hypoallergenic ingredients, and it is well known that extensively hydrolysed infant formula which have little intact protein and a peptide size distribution <1 200 Da have reduced allergenicity *in vivo* in allergic infants (Dupont *et al.*, 2015; Giampietro *et al.*, 2001; Nutten *et al.*, 2020). Such data suggest that short peptides in hydrolysates are poorly allergenic, and hence hydrolysates will have increased RfDs. However, there is a lack of data in this regard, and it is unclear if the analytical workflows developed for monitoring hypoallergenicity could provide a means to address analytical requirements for detection of hydrolysates more generally. While extensively hydrolysed infant formula are recognized as hypoallergenic and display reduced allergenicity, a small number of infants have been clinically described who are reactive to ingestion of these formulae (Saylor and Bahna, 1991; Chauveau *et al.*, 2016; Flores and Persaud, 2020).

Cross-reactivity issues: An important aspect of allergen test methodology is to ensure that it is specific, which can be challenging in some instances due to the close sequence similarity of allergens from different sources. This can lead to false positive test results in terms of the specific species detected, as exemplified by the issue of apparent residues of almond in samples of paprika and cumin detected by ELISA which resulted in a product recall in the United Kingdom. On further investigation, cross-reactivity issues were identified between almond and kernels from other *Prunus* species including *Prunus mahaleb*. The presence of *Prunus mahaleb* and not almond (*Prunus dulcis*) was confirmed using a combination of MS and DNA based methods (Walker *et al.*, 2018), and the product recall was rescinded in the United Kingdom, although interestingly mahaleb has subsequently been shown to be an IgE cross-reactive spice in tree nut allergic subjects (Noble *et al.*, 2017) with a case report describing clinical reactivity to mahaleb in an individual with multiple tree nut allergies, including almond (Benoit *et al.*, 2020).

Specificity issues are particularly difficult to address in relation to detection of fish, crustacea and wheat and are discussed in more detail below. The broad specificity issue influences the detection and quantification capability of fish and crustacean ELISAs. It is also lack of knowledge on specificity of test methods for wheat, as opposed to detection of cereals containing gluten is unclear.

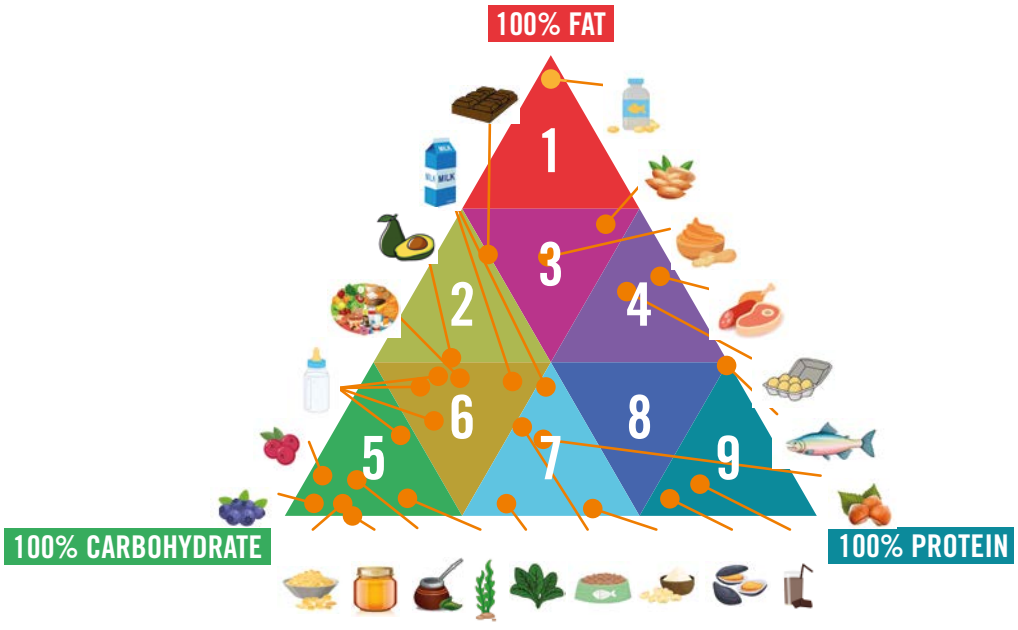
- ***Fish:*** Of some 34 300 fish species, in excess of 1 000 members are currently commercialized and consumed worldwide. Except for a few countries which specify certain fish species for allergen control (e.g. the Republic of Korea and Japan), most countries retail fish and related products without limiting their allergen labelling to defined species. ELISAs developed for the detection of fish protein residues in foods generally target parvalbumin, the major fish allergen, from a very few commonly consumed species associated with a high frequency of clinical reactions such as cod, or from demonstrated allergenicity such as silver carp and Pacific mackerel (Sørensen *et al.*, 2017; Bugajska-Schretter *et al.*, 1998; Hamada *et al.*, 2003). Another immunogen target for ELISA development is heat-stable proteins such as sarcoplasmic protein (catfish) (Ruethers *et al.*, 2018). Parvalbumins from different fish species show diverse amino acid sequences. The WHO-IUIS registered β-parvalbumins in fish show sequence identities ranging from 51–99 percent (Ruethers *et al.*, 2018). Nevertheless, both polyclonal and monoclonal antibodies have been raised against parvalbumins either from a single species or a mixture of selected species (Cai *et al.*, 2013; Chen and Hsieh, 2014; Fæste and Plassen, 2008; Gajewski *et al.*, 2009; Shibahara *et al.*, 2013; Weber *et al.*, 2009, Liang *et al.*, 2022). Only a limited number of fish ELISA test kits (e.g. AgraQuant Fish ELISA kit from Romer Labs [based on the anti-cod parvalbumin polyclonal antibody] and Common Bone Fish Antigen ELISA kit from XEMA [based on the anti-cod tropomyosin complex polyclonal antibody]) are available commercially. Both polyclonal and monoclonal antibodies detect and quantify their antigens (derived from their immunogens) well, yielding acceptable recoveries for some fish species (Fæste and Plassen, 2008; Shibahara *et al.*, 2013; Cai *et al.*, 2013; Liang *et al.*, 2022). The primary challenge of the fish ELISAs is their broad specificity to different fish species, which varies widely depending on the fish species used in the preparation of immunogens and how the assays have been developed and formatted (Liang *et al.*, 2022). The way cross-reactivity between species is determined is also different between assays, making impossible direct comparison between assays. Commercial test kits tend to detect common fish species from common European and North American origins but exhibit limited capacities to detect fish species from the Asia-Pacific region (Ruethers *et al.*, 2020). Although some assays showed detection of fish residues in fermented foods such as fish sauce (Liang *et al.*, 2022), food matrices containing highly hydrolysed fish proteins would still pose an analytical challenge. Other matrices such as wine and soy sauce showed poor recoveries due to the presence of polyphenols and other interfering substances (Fæste and Plassen, 2008; Cai *et al.*, 2013).

- ***Crustacea:*** Crustaceans can be divided into prawns/shrimps, crabs, lobsters, crayfish, krill, and barnacles. Most development efforts for crustacean ELISAs have been devoted to the detection of prawn and shrimp allergens due to the popularity of, and high exposure to, those foods. The protein target for crustacean ELISAs is tropomyosin, the major allergen in shellfish. The amino acid sequence similarity of tropomyosin within the crustacean group is 88–100 percent and 98–100 percent for prawns (Reese *et al.*, 1999; Lopata, O'Hehir and Lehrer, 2010). Both polyclonal and monoclonal antibodies have been raised against tropomyosin purified from selected prawn/shrimp species such as black tiger prawn (Seiki *et al.*, 2007) and caridean shrimp (Klotz, Karge and Von Rintelen, 2007). However, many studies lack validation with food matrices. As for fish ELISAs, crustacean (prawn) ELISAs also show broad specificity to different prawn and shrimp species, lobsters and crabs within the order *Decapoda* and very low to negligible cross-reactivity to species belonging to other crustacean orders.
- **Determination of wheat in food compared to cereals containing gluten:** The determination of residual gluten in gluten-free foods is described in the CODEX recommendation (FAO and WHO, 2015). Gluten is a protein fraction of wheat and related species comprising the seed storage prolamins which are known to be toxic to people with coeliac disease. Many of the allergens implicated in IgE-mediated allergy to wheat include seed storage prolamins found in gluten, notably ω-5 gliadin, an allergen considered of particular clinical relevance in wheat-dependent exercise-induced anaphylaxis (Kennard *et al.*, 2018) together with an LMW glutenin subunit allergen Tri a 36 (Baar *et al.*, 2012) and a 1Bx-type HMW subunit of glutenin (Baar *et al.*, 2014), although other allergens include members of both the α- and γ-gliadin fractions (Mameri *et al.*, 2015). Like coeliac toxic motifs, the IgE epitopes identified in these proteins are found in the repetitive domain of the seed storage prolamins (Sollid *et al.*, 2020; Juhász *et al.*, 2018). Other wheat allergens are non-gluten proteins including the lipid transfer protein (LTP) and purothionin, amongst others (Pahr *et al.*, 2014; Pastorello *et al.*, 2007). Current ELISA tests methods for determination of gluten employ antibodies which are not wheat specific, recognizing seed storage prolamins from wheat, rye and barley (Lexhaller, Tompos and Scherf, 2017). Indeed, the R5 antibody used in the first assays reliably capable of detecting <20 ppm gluten was raised against ω-secalin from rye (Hochegger, Mayer and Prochaska, 2015). It is therefore unsurprising that they do not recognize the non-gluten protein allergens implicated in IgE-reactivity to wheat. Similarly, many of the peptide markers being used in the development of MS methods for determination of gluten in food are found in several cereal species and are not wheat specific (Henrottin *et al.*, 2019; Seki *et al.*, 2021). However, similar cross-reactivity is observed in IgE responses in humans with IgE cross-reactivity having been demonstrated between ω-5 gliadin, the γ-secalins from rye and the γ-3 hordein from barley (Palosuo *et al.*, 2001), and patients are usually advised to adhere to a gluten/wheat free diet (Zubrinich *et al.*, 2021).

Thus, it will be necessary to evaluate current test methods available for determining gluten in foods in order to confirm their utility for measuring wheat in food, both in terms of the test's specificity in light of clinical practice, and in terms of advice to patients. A comparison of gluten ELISA methods found "a qualitative response model revealed that there is a 50% probability that a food product deemed compliant at the 20 mg/kg threshold on the basis of measurements performed by commercial ELISA test kits may, in reality, contain up to 80–90 mg/kg" (Rzychon *et al.*, 2017). As a result, a food product deemed compliant at the 20 mg/kg level applicable to foods claimed to be gluten-free may, in reality, only meet the RfD (5 mg total wheat protein) for intakes of up to 50 g rather than the 250 g calculated from the gluten-free limit (see Table 15).

Method validation: Analytical methods should be validated to demonstrate that they are suitable for their end use (i.e. that they have the intended specificity, accuracy and precision). Since methods are needed to detect and quantify allergens in a variety of food matrices, it is essential that they are evaluated for performance in each food matrix (or group/type of food matrix) using approaches such as the AOAC triangle (Figure 8). This triangle was developed by the AOAC International's Task Force on Methods for Nutrition Labelling to classify foods based on their fat, protein and carbohydrate content, one or two foods within a sector being considered representative of other foods in that sector.

FIGURE 8. **AOAC FAT-PROTEIN-CARBOHYDRATE TRIANGLE. EACH SECTOR (NUMBERED 1–9) REPRESENTS DIFFERENT TYPES OF A FOOD MATRIX. ORIGINALLY DEVELOPED TO PROVIDE A FRAME OF REFERENCE FOR ANALYSIS OF NUTRIENTS, THIS HAS BEEN USED MORE WIDELY INCLUDING FOOD-MATRIX REFERENCE MATERIALS.**



Source: Reprinted courtesy of NIST. All rights reserved, US Secretary of Commerce. Sharpless *et al.*, 2014. **Sharpless, K.E., Lippa, K.A., Duewer, D.L. & Rukhin, A.L.** 2014. The ABCs of using standard reference materials in the analysis of foods and dietary supplements: a practical guide. Available at: https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-181.pdf

To evaluate matrix effects, foods can be spiked in the first instance with known amounts of allergen reference material to determine recovery. Whenever possible, validation of allergen test methods should include analysis of allergen-incurred food samples since processing can profoundly affect allergen recovery, detection and quantitation. Incurred quality control and reference materials need to contain allergens at concentrations both at and below the proposed action level for a given matrix and be able to support routine monitoring of test method LoQ. Due to the importance of method validation and the impact of food matrices on test method LoQ, an assessment of test methods for selected allergenic ingredients in matrices that represent different sectors of the AOAC triangle was undertaken (Table 15). These were baked goods (such as bread and cookies) that occupy sector 5 of the triangle and chocolate, which occupies sector 2. Both are known to present challenges to allergen test methods. The review was undertaken to identify whether test methods were potentially available that could quantify allergenic ingredients at the action levels calculated in Tables 11 or 12. This review found that test method LoQs were sufficiently low to allow analysis to support action levels in such matrices when they were based on HBGVs derived from the ED_{05}, but not the ED_{01}, level and a reference amount (food consumption) based on the P75.

Test method reporting units and reference materials: Since HBGV have been derived in terms of total protein from the allergenic source (food, ingredient), conversion factors must be applied or calibrators and reference materials used which can allow test methods to report allergen analysis in terms of mg protein from the allergenic source/kg food product. Reference materials have been shown to support harmonization of gluten determination using different ELISA methods (Rzychon *et al.*, 2017), and it has been acknowledged that reference materials are needed to improve quantitative reporting of allergens in food, but such materials have been lacking (Walker *et al.*, 2016). International efforts have been underway for some years to fill these gaps (Dumont *et al.*, 2010), and ISO 17034 accredited materials are now available for some allergens in a chocolate matrix (LGC, 2021) and the National Institute of Standards and Technology (NIST) Food Allergen Program (NIST, 2021). However, the wider availability of such materials for priority food allergens, both as ingredients and as materials incurred into food matrices where unintended allergen presence can be more common, will be essential to harmonize test method results and integrate them with HBGV proposed for allergens in foods, as well as provide a means for ongoing monitoring of test method LoQ.

Sampling: There are no validated allergen sampling plans, and there are issues over the way in which testing laboratories are provided with samples. Often, they are neither taken, stored nor shipped appropriately, and appropriate information about sample handling and care is often not provided to the analytical laboratory. Guidance on how to take and homogenize samples would be valuable.

Sampling of particulates also presents particular problems. The chance that a particle of given size and weight would end up in a product and might be consumed was modelled in the iFAAM project for some defined scenarios. Initially, the size and weight of a particle that might represent the reference dose of peanut protein was assessed ;

one peanut particle of 0.5 mm in size could contain enough protein to exceed the reference dose at ED_{01}/ED_{05}. Similarly, with sesame seeds, each one contains 3.5–7 mg of protein, which is again in excess of the reference dose at ED_{01}/ED_{05}. When considering skimmed milk powder, a lump of powder of 0.35–0.75 mm contains sufficient milk protein to exceed the reference dose at ED_{01}/ED_{05}. Whether an individual reacted would then depend on their individual threshold of reaction.

The expert committee discussed the issues that sampling limitations and lack of defined sampling plans raised for analysis. It was clear that designing a sampling plan for homogeneously distributed allergens was demanding enough, while for particulate contamination it could be like "looking for a needle in a haystack", and that was not a practical path to follow. It was also agreed that there needed to be a definition of a particulate and where the cut-off is with, for example, dust. Large lumps and parts of finished product breaking off, cookies for instance, and then finding their way into another food product also posed problems for evaluation. It was agreed that there is a need to evaluate sampling plans, but it was concluded by the group that particulates are out of scope as a consideration related to the performance of analytical methods. However, particulate contamination was highly pertinent to the third meeting of this *ad hoc* consultation. The discussions at the third meeting would also need to consider issues relating to dust and how this needs to be addressed. "Dust" requires a definition – for example, one that might be adopted is "airborne particulates". According to Safeopedia, "dust, in the context of occupational health and safety, refers to suspended organic or inorganic particles in the atmosphere" (Safeopedia Inc., 2018). The OECD has also adopted a definition from the UN: "Dust refers to particles light enough to be suspended in air" (UN, 1997). The discussions could refer to good hygiene practices in relation to dust management and scenarios where it might be a hazard and result in a PAL being applied.

Lack of ISO 17925-Certified laboratories qualified to conduct allergen assays also hinders development and harmonization in this field.

8.3 CONCLUSIONS

The reference doses (RfD) recommended in this report can be implemented and monitored to some degree with current analytical capabilities. However, users of these services need to understand that all current methods have significant limitations and need to allow for these when interpreting and using results.

- General analytical considerations pertaining to the application of reference doses (RfD):
  - Operational use of RfD (mg of total protein from the allergenic source) requires their conversion to action levels (mg of total protein from the allergenic source per kg of food), based on data on food consumption/intake per eating occasion to monitor adherence to the established RfD.

- Consumption quantities should be based on a percentile consumption estimate per single eating occasion appropriate to the intended protection level.
- Test method reporting units should be harmonized by expressing them in mg total protein from the allergenic source/kg of food containing the putative allergen unintentionally.
- Method performance criteria indicate that the limits of quantification (LoQ) of any method utilized for a specific food should be around 3-fold lower than the action level for that food in order to account for real-world performance variability and to assure that the analytical result is truly at or below the action level.
- An initial assessment of test methods for selected priority allergenic foods in food matrices such as baked goods and chocolate suggest that they have sufficient sensitivity to quantify allergens at the action levels set using an HBGV based on the ED_{05} and a reference amount (food consumption) based on P75.

- Known current deficiencies and/or inconsistencies in analytical methodologies and methods. These include but are not limited to:
  - lack of methods that are fit for purpose in identification and quantification of many priority allergens. Few test methods provide information on specificity, and many lack sufficient data on validation, especially with regards to quantification of the analyte in food matrices, complicating the choice of appropriate test methods by analytical laboratories;
    - Specificity issues in relation to fish, crustacea and wheat are of particular concern as fit for purpose test methods are largely lacking for these priority allergens.
  - limited availability of reference materials and absence of reference methods;
  - poor recovery or ability to extract proteins from complex food matrices and lack of validation in a sufficient diversity of food matrices;
  - poor recovery of proteins from food matrices as a result of processing unit operations including thermal processing and fermentation; and
  - need to develop or adapt sampling plans to facilitate the monitoring of adherence to an established RfD.
- Where analytical capability (test performance) is insufficient to apply action levels that can be used to monitor adherence to a recommended RfD, a temporary action level could be set at a higher level (at analytical limit) while awaiting improved methods. The RfD can help steer the improvement of methods as these provide the performance criteria needed. The full range of ED values, such as those published in Houben *et al.* (2020), can help assess the risk associated with such a temporary higher action level.

TABLE 15 **ASSESSMENT OF TEST METHOD PERFORMANCE FOR SELECTED ALLERGENIC FOODS IN EXEMPLAR MATRICES REPRESENTING DIFFERENT SECTORS OF THE AOAC TRIANGLE (CF. FIGURE 8)**

ALLERGENIC FOOD INGREDIENT	MATRIX	INTAKE CATEGORY (Table 14 P75s rounded up to nearest 10 g increment)	PROPOSED ACTION LEVEL (Table 12) and desired method LoQ (Table 13) (µg protein/ g food) (mg protein/kg food)	METHOD AND LOQ REPORTED BY MANUFACTURERS/ RESEARCHERS (including reporting units)	REFERENCES	COMMENTS
Milk	Cookies/ biscuits	50 g	Proposed action level: 40 Desired method LoQ: 13.3	Several commercial ELISA methods, see comments for details	Khuda *et al.* (2012a) Weiss *et al.* (2016)	Khuda *et al.* (2012a) found that several commercial milk ELISA kits were able to detect casein at the LoQs claimed by these manufacturers in a cookie dough matrix. However, several kits (Morinaga and R-Biopharm) were not able to detect casein at the LoQ values provided by the kit manufacturers. Based on the recoveries of milk in incurred baked cookies, many of the evaluated kits were not able to detect milk at the LoQ specified by the kit manufacturers and thus would not be able to detect at the LoQ for ALs based on the ED_{01} level, but some would be able to detect at the LoQ for ALs based on the ED_{05} level. Weiss *et al.* (2016) reported that RIDASCREEN (R) FAST Milk Kit was able to detect at the method LoQ (2.5 mg milk protein/kg) when cookies were spiked with milk (cookies were not incurred). Based on this validation study, this kit would not be able to detect milk protein at the LoQ for ALs based on the ED_{01} but would be sufficient for detecting it at ALs based on the ED_{05} LoQ value.
	Chocolate	40 g	Proposed action level: 50 Desired method LoQ: 16	Several commercial ELISA methods, see comments for details.	Khuda *et al.* (2012b) Weiss *et al.* (2016)	All commercial ELISA kits evaluated by Khuda *et al.* (2012b) detected casein at the manufacturers' claimed LoQs using a dark chocolate matrix, but the quantitated levels were greatly overestimated. According to Weiss *et al.* (2016), the RIDASCREEN FAST Milk Kit was able to detect at the method LoQ (2.5 mg milk/kg) when chocolate dessert was spiked with milk (dessert was not incurred). Based on this validation study, this kit would not be able to detect milk protein at the LoQ for ALs based on the ED_{01} but would be sufficient for detecting the LoQ at ALs based on the ED_{05}.
Egg	Cookies/ biscuits	50 g	Proposed action level: 40 Desired method LoQ: 13.3	Several commercial ELISA methods, see comments for details	Khuda *et al.* (2012a)	Khuda *et al.* (2012a) reported that all ELISA kits evaluated were able to detect egg at the claimed LoQs in the cookie dough matrix. In contrast, none of the kits were able to detect egg protein at the kit LoQs in cookies baked for 30 min. All kits were able to detect egg at the three highest incurred levels (12, 48, and 240 µg /g egg protein) after baking, but at measured levels that were considerably lower than the calculated incurred levels. Based on this study, the ELISA kits would not be able to detect egg protein at the LoQ for ALs based on the ED_{01} level but would be sufficient for detecting egg protein at the LoQ for ALs based on the ED_{05} level.
Peanut	Chocolate	40 g	Proposed action level: 50 Desired method LoQ: 16	Several commercial ELISA methods; see comments for details.	Khuda *et al.* (2012b)	Khuda *et al.* (2012b) reported that on the basis of recoveries at the lowest incurred levels (1.26 ug peanut protein/g chocolate) several ELISA kits (MO, ES, and TE) kits were not able to detect peanut protein in these chocolate samples at the limits of quantitation (LoQs) claimed by the manufacturer. Only the R-Biopharm (RB) kits detected peanut protein close to the kit LoQ in chocolate (tempered). Based on this study, most kits would not be able to detect peanut protein in chocolate at the LoQ for ALs based on the ED_{01}, but most would be able to detect at the LoQ for ALs based on the ED_{05}.
Almond	Chocolate, ice cream, cookies, pasta sauce	40 g for chocolate, 50 g for cookies, 80 g for sauce and 100 g for ice cream	**Chocolate** Proposed action level: 25 Desired method LoQ: 8.3 **Cookies** Proposed action level: 20 Desired method LoQ: 6.6 **Sauce and ice cream** Proposed action level: 10 Desired method LoQ: 3.3	ELISA method for almond: LoQ quoted as 2.5 almond mg/kg which equates to 0.53 mg almond protein/ kg (assuming almond contains 21% protein)	Slotwinski *et al.* (2018)	Authors used foods spiked with almond paste foods rather than incurred foods to determine recoveries at 0, 5 and 10 ug almond levels (0, 1.05, and 2.10 µg almond protein/g levels). Recoveries were between 73 and 110% for the spiked almond. The ELISA is sensitive enough to detect almond protein at the LoQ for ALs based on the ED_{05}, but not the ED_{01}.

TABLE 15 **ASSESSMENT OF TEST METHOD PERFORMANCE FOR SELECTED ALLERGENIC FOODS IN EXEMPLAR MATRICES REPRESENTING DIFFERENT SECTORS OF THE AOAC TRIANGLE (CF. FIGURE 8)** (continued)

ALLERGENIC FOOD INGREDIENT	MATRIX	INTAKE CATEGORY (Table 14 P75s rounded up to nearest 10 g increment)	PROPOSED ACTION LEVEL (Table 12) and desired method LoQ (Table 13) (µg protein/ g food) (mg protein/kg food)	METHOD AND LOQ REPORTED BY MANUFACTURERS/ RESEARCHERS (including reporting units)	REFERENCES	COMMENTS
Hazelnut	Bread roll	120 g	Proposed action level: 25 Desired method LoQ: 8.3	MS method LoQ: Used 1 peptide marker from Cor a9 LNALEPTNR; LoQ quoted in paper was <10 mg/kg	Korte and Brockmeyer (2016)	The method does not have the sensitivity to detect hazelnut protein at the LoQ for ALs based on the ED_{01} but is able to detect hazelnut protein at the LoQ for ALs based on the ED_{05}.
	Chocolate, tomato sauce, cookie and ice cream	40 g for chocolate, 50 g for cookies, 80 g for sauce and 100 g for ice cream	**Chocolate** Proposed action level: 75 Desired method LoQ: 25 **Cookies** Proposed action level: 60 Desired method LoQ: 20 **Sauce** Proposed action level: 35 Desired method LoQ: 11 **Ice cream** Proposed action level: 30 Desired method LoQ: 10	MS Method: used 6 peptide markers; LoQ for peptides ranged from 2.5–12.5 ug/g; quoted an LoQ of 2.5 mg hazelnut protein/kg	Planque *et al.* (2017)	1. Heavy peptides were not used to calculate recoveries; the indicated LoQ is based on SIDs and not on calculating recoveries from analysed samples and have been derived using signal-to-noise ratios using incurred samples. 2. High coefficient of variations (CVs) returned – often seen in MS methods due to laborious sample preparation 3. Matrix interference observed in cookies and chocolate rather than in ice cream and sauce, and tannins in chocolate The method does not have the sensitivity to detect hazelnut protein at the LoQ for ALs based on the ED_{01} but is able to detect hazelnut protein at the LoQ for ALs based on the ED_{05}.
	Chocolate	40 g	Proposed action level: 75 Desired method LoQ: 25	Sandwich ELISA method: LoQ quoted was 50 mg hazelnut/kg or 7 mg hazelnut protein/kg	Costa *et al.* (2015)	The method does not have the sensitivity to detect hazelnut protein at the LoQ for ALs based on the ED_{01} but is able to detect hazelnut protein at the LoQ for ALs based on the ED_{05}.
Walnut	Chocolate, cookie, ice cream and sauce	40 g for chocolate, 50 g for cookies, 80 g for sauce and 100 g for ice cream	**Chocolate** Proposed action level: 25 Desired method LoQ: 8.3 **Cookies** Proposed action level: 20 Desired method LoQ: 6.6 **Sauce and ice cream** Proposed action level: 10 Desired method LoQ: 3.3	MS method: two peptide markers, LoQ for peptides ranged from 5–10 ug/g; quoted an LoQ of 5 mg walnut protein/kg, one of which was ATLTLVSQETR derived from Jug r 2 the 7S globulin (Uniprot accession Q9SEW4)	Planque *et al.* (2017)	1. No isotopically labelled peptides were used to calculate recoveries; the indicated LoQ is based on SIDs and not on calculating recoveries from analysed samples and have been derived using signal: noise ratios using incurred samples. 2. High coefficient of variations (CVs) returned – often seen in MS methods due to laborious sample preparation 3. Matrix interference observed in cookies and chocolate rather than in ice cream and sauce, and tannins in chocolate The method does not have the sensitivity to detect walnut protein at the LoQ for ALs based on the ED_{01} but is able to detect walnut protein at the LoQ for ALs based on the ED_{05} for some, but not all, food categories.
	Bread roll	120g	Proposed action level: 8 Desired method LoQ: 2.6	MS method: one peptide marker ATLTLVSQETR <3 mg/kg	Korte and Brockmeyer (2016)	Sensitivity is lacking to allow the test method to quantify at the LoQ for ALs based on either the ED_{01} or ED_{05}.
Pecan	Chocolate, cookie, ice cream and sauce	40 g for chocolate, 50 g for cookies, 80 g for sauce and 100 g for ice cream	**Chocolate** Proposed action level: 25 Desired method LoQ: 8.3 **Cookies** Proposed action level: 20 Desired method LoQ: 6.6 **Sauce and ice cream** Proposed action level: 10 Desired method LoQ: 3.3	MS method: four peptides markers, LoQ for peptides ranged from 5–25 ug/g; quoted an LoQ of 5 mg pecan protein/kg	Planque *et al.* (2017)	See comments for walnut above.

TABLE 15 **ASSESSMENT OF TEST METHOD PERFORMANCE FOR SELECTED ALLERGENIC FOODS IN EXEMPLAR MATRICES REPRESENTING DIFFERENT SECTORS OF THE AOAC TRIANGLE (CF. FIGURE 8)** (continued)

ALLERGENIC FOOD INGREDIENT	MATRIX	INTAKE CATEGORY (Table 14 P75s rounded up to nearest 10 g increment)	PROPOSED ACTION LEVEL (Table 12) and desired method LoQ (Table 13) (µg protein/ g food) (mg protein/kg food)	METHOD AND LOQ REPORTED BY MANUFACTURERS/ RESEARCHERS (including reporting units)	REFERENCES	COMMENTS
Cashew	Chocolate, cookie, ice cream and sauce	40 g for chocolate, 50 g for cookies, 80 g for sauce and 100 g for ice cream	**Chocolate** Proposed action level: 25 Desired method LoQ: 8.3 **Cookies** Proposed action level: 20 Desired method LoQ: 6.6 **Sauce and ice cream** Proposed action level: 10 Desired method LoQ: 3.3	MS method: four peptide markers all with an LoQ of 2.5 µg/g; quoted an LoQ of 2.5 mg cashew protein/ kg, one of which was ADIYTPEVGR derived from Ana o 2 (Uniprot accession no Q8GZP6)	Planque *et al.* (2017)	See comments for walnut above regarding test method development. **None of the peptide markers are able to quantify at the LoQ for ALs based on the ED_{01}, but they are able to achieve required sensitivity at the LoQ for ALs based on ED_{05}.**
	Bread roll	120 g	Proposed action level: 8 Desired method LoQ: 2.6	MS method: ADIYTPEVGR LoQ <0.3mg/kg	Korte and Brockmeyer (2016)	Full validation using isotopically labelled peptide markers. Data are lacking to identify whether the test method can quantify at the LoQ for ALs based on either the ED_{01} or ED_{05}.
Pistachio	Chocolate, cookie, ice cream and sauce	40 g for chocolate, 50 g for cookies, 80 g for sauce and 100 g for ice cream	**Chocolate** Proposed action level: 25 Desired method LoQ: 8.3 **Cookies** Proposed action level: 20 Desired method LoQ: 6.6 **Sauce and ice cream** Proposed action level: 10 Desired method LoQ: 3.3	MS method: five peptide markers all with a LoQ of 2.5 ug/g; quoted an LoQ of 2.5 mg pistachio protein/kg, one of which is AMISPLAGSTSVLR from the 11S globulin allergen Pis v 5 (Uniprot accession no B7SLJ1)	Planque *et al.* (2017)	See comments for walnut above regarding test method development. **None of the peptide markers are able to quantify at the LoQ for ALs based on the ED_{01}, but they are able to achieve required sensitivity at the LoQ for ALs based on the ED_{05}.**
	Bread roll	120 g	Proposed Action Level: 8 Desired method LoQ: 2.6	MS method: AMISPLAGSTSVLR LoQ 1mg/kg	(Korte and Brockmeyer, 2016)	Full validation using isotopically-labelled peptide markers. **The peptide reporter and method can only quantify with sufficient sensitivity at the LoQ for ALs based on the ED_{05} level.**
Wheat	Cookies, fruit compote	Cookies 50 g	**Cookies** Proposed Action Level: 100 Desired method LoQ: 33	MS method: two peptide markers - Wheat, spelt, kamut and einkorn NYLLQQCNPVSLVSSLVSMILPR, LoD only 50mg/kg Wheat, spelt and kamut: VFLQQQCSPVAMPQSLAR, LoD only 10mg/kg	(Henrottin *et al.*, 2019)	
Wheat determined as gluten	Infant semolina	200 g	Proposed Action Level: 25 Desired method LoQ: 8.3	Various ELISA test kits evaluated through proficiency testing 2007–2011, see comments for details	(Scharf *et al.*, 2013)	Wheat semolina, whole flour, wheat whole grain flakes, spelt, and oat flour were mixed with infant cereal semolina comprising sorghum and rice. ELISAs tested at the lowest level of 7.5 mg gluten/kg. Although in general the ELISA test kits were able to detect the presence of gluten, the recoveries varied greatly – from 3.4–200% depending on the nature of the gluten added and the test kit used. **None of the test methods had LoQs that would allow determination of wheat as gluten at ALs based on the ED_{01}; however, ALs based on the ED_{05} are near or above that required for gluten-free foods (20 mg/kg) and would be faced with the same issues of reliability faced by standard gluten analysis.**

TABLE 15 **ASSESSMENT OF TEST METHOD PERFORMANCE FOR SELECTED ALLERGENIC FOODS IN EXEMPLAR MATRICES REPRESENTING DIFFERENT SECTORS OF THE AOAC TRIANGLE (CF. FIGURE 8)** (continued)

ALLERGENIC FOOD INGREDIENT	MATRIX	INTAKE CATEGORY (Table 14 P75s rounded up to nearest 10 g increment)	PROPOSED ACTION LEVEL (Table 12) and desired method LoQ (Table 13) (µg protein/ g food) (mg protein/kg food)	METHOD AND LOQ REPORTED BY MANUFACTURERS/ RESEARCHERS (including reporting units)	REFERENCES	COMMENTS
Fish	Wine and soy sauce	283 g for wine; 30 g for soy sauce	**Wine** Proposed action level: 15 Desired method LoQ: 5 **Soy sauce** Proposed action level: 150 Desired method LoQ: 50	ELISA Polyclonal against cod parvalbumin: 5 mg/kg (buffer blank mean value plus 10 times the standard deviation [SD] of the mean value)	Fæste and Plassen (2008)	According to the reported LoQ, the assay can quantify at ALs based on the ED_{05} for all the tested matrices, but not at ALs based on the ED_{01} for wine; it should be noted that there are several potential issues including: fish species specificity with variable detection (poor detection against sturgeon, Northern pike, and anchovy); and poor recovery from soy sauce (36–87%) and wine (14–40%). The assay was sensitive to Ca2+-concentration in the assay buffer.
	Chicken meatball; creamy croquette; pork meatball dumpling; vegetable and chicken soup; rice gruel	126 g of chicken meatball and pork meatball dumpling; 180 g creamy croquette; 200 g of rice gruel; 400 g of vegetable and chicken soup	**Chicken/pork meatball dumpling** Proposed action level: 35 Desired method LoQ: 11 **Vegetable and chicken soup** Proposed action level: 10 Desired method LoQ: 3.3	ELISA Polyclonal against Pacific mackerel parvalbumin): 0.70 µg fish protein/g in food	Shibahara *et al.* (2013)	According to the LoQ reported, the assay can detect at ALs based on the ED_{01} and ED_{05}. The cross-reactivity of seven fish species relative to the immunogen indicated 22.6–99% cross-reactivity. The assay cannot measure fish protein in fermented foods such as fish sauce and shuto, although detection of hydrolysed fish protein in fish sauce is noted. Some cross-reactivity with cephalopods and fish roes resulted in false positives.
	Tofu soup; mushroom soup; soy sauce	400 g of Tofu soup; mushroom soup; 30 g for soy sauce	**Tofu soup and mushroom soup** Proposed action level: 10 Desired method LoQ: 3.3 **Soy sauce** Proposed action level: 150 Desired method LoQ: 50	ELISA Monoclonal against silver carp parvalbumin: 0.3 mg/kg (buffer blank mean value minus ten times the standard deviation (SD) of the blank value)	Cai *et al.* (2013)	According to the LoQ reported, the assay can detect at the proposed ALs based on the ED_{01} and ED_{05}. mAb B2-E1 cross-reacted with frog parvalbumin, but very limited cross-reactivity was studied with fish species (silver carp, common carp, tilapia, blueround scad, pollock). Recovery of carp parvalbumin in soy sauce, however, was poor.
	Almond coconut muesli; chicken corn soup; chicken corn soup	60 g of muesli; 400 g of chicken corn soup & chicken corn soup	Muesli: 80 Desired method LoQ: 26 Chicken corn soup & chicken corn soup: 10 Desired method LoQ: 3.3	ELISA based polyclonal antibody against mixed species: 0.007 mg protein/kg (buffer blank mean value minus ten times the standard deviation [SD] of the blank value)	Liang *et al.*, 2022	According to the LoQ derived from LoD reported, the assay can detect at ALs based on the ED_{05} and potentially at ALs based on the ED_{01}. Of 37 fish species tested, the ELISA could only detect 28 fish species (76%). The assay could detect fish residues in highly processed foods containing fish as an ingredient, such as fermented fish seasoning powder, fermented fish extract, curry sauce and sour fish sausage
				Three commercial ELISAs (Romer labs and XEMA) ELISA Romer lab: 4 ppm cod	Ruethers *et al.* (2020)	AgraQuant Fish ELISA kit from Romer Labs – polyclonal antibody against parvalbumin from cod; Common Bone Fish Antigen ELISA kit from XEMA – polyclonal against muscular tropomyosin complex from cod. The tested ELISAs detected better common European and North American species, but limited capacity to detect fish species from Asia-Pacific region.
Crustacean shellfish	Fish ball sausages; freeze-dried egg soup; chicken meatball	130 g for chicken meatball; 150 g for fish ball sausages; 400 g for freeze-dried egg soup	**Chicken/pork meatball dumpling** Proposed action level: 1500 Desired method LoQ: 500 **Fish ball sausages** Proposed action level: 1000 Desired method LoQ: 333 **Soup** Proposed action level: 500 Desired method LoQ: 166	ELISA Poly- and monoclonal antibodies against black tiger prawn tropomyosin: 0.66 mg protein/kg of food sample	Sakai *et al.* (2008); Koizumi *et al.* (2014)	According to the LoQ reported, the assay is capable of detecting ALs based on both ED_{01} and ED_{05} for certain members of crustacean shellfish, but not all. The cross-reactivity to other prawn/shrimp, lobster and crab within the *Decapoda* group varied between 47–110%. Cross-reactivity with other members of crustacean shellfish is <1.8% (determined at 50% response concentration relative to black tiger prawn). The recovery of black tiger prawn protein in the model foods (listed in the Matrix column) is 84–101%. Other prawn proteins in food matrices were not tested.

Source: Authors' own elaboration.

CHAPTER 9
DEFINITION OF REFERENCE DOSES (RfDs)

The process followed to derive RfDs is described in detail in Section 5 (Translating clinical data into reference doses as health-based guidance values [HBGV], and operational risk management practice). Briefly, the first step, which took place in plenary, consisted of reviewing both discrete and cumulative ED_{01} and ED_{05} values for the priority allergens, considering the potential biases which could affect how representative the values are and confirming that they were appropriate as the basis for the work of the hazard characterization group.

The hazard characterization group then considered the characteristics of reactions following exposure to amounts of total allergenic protein corresponding to ED_{01} and ED_{05}. They presented their findings and conclusions to the whole expert committee to inform the next stage, including the final formulation of the proposed RfDs.

9.1 RECOMMENDATION FOR INDIVIDUAL ALLERGEN REFERENCE DOSE BASED ON THE ELICITING DOSE PREDICTED TO PROVOKE REACTIONS IN 5 PERCENT OF THE ALLERGIC POPULATION (ED_{05})

The ED_{05} was recommended for further RfD derivation based on hazard characterization and as defined by the safety objective to:

> minimise, to a point where further refinement does not meaningfully reduce public health impact, the probability of any objective allergic response, as defined by dose-distribution modelling of minimum eliciting doses (MEDs) and supported by data regarding severity of symptoms in the likely range of envisioned Reference Doses (RfD).

Table 16 lists the complete set of discrete and cumulative ED_{05} values for further derivation of RfDs.

TABLE 16 **FOOD-ALLERGIC POPULATION ELICITING DOSES (EDS)**

	DISCRETE ED_{05} (95% CI)	CUMULATIVE ED_{05} (95% CI)
CASHEW	0.8 (0.2, 5.0)	1.6 (0.4, 9.4)
EGG	2.3 (1.2, 4.7)	2.4 (1.3, 5.3)
FISH	12.1 (4.5, 43.9)	15.6 (4.6, 102)
HAZELNUT	3.5 (1.3, 12.1)	4.7 (1.7, 15.7)
MILK	2.4 (1.3, 5.0)	3.1 (1.6, 6.6)
MILK, BLOM *ET AL.* (2022)	3.2 (1.8, 6.4)	4.3 (2.4, 9.0)
PEANUT	2.1 (1.2, 4.6)	3.9 (2.8, 7.1)
SESAME	2.7 (0.4, 33.6)	4.2 (0.6, 57.7)
SESAME, TURNER *ET AL.* (2022C)	2.4 (1.0, 7.7)	2.5 (0.9, 9.5)
SHRIMP	280 (69.3, 880)	429 (94.0, 1854)
WALNUT	0.8 (0.1, 8.9)	1.2 (0.1, 13.0)
WHEAT	6.1 (2.6, 15.6)	9.3 (3.9, 24.9)

Source: Reproduced from Remington *et al.* (2020) unless otherwise noted. **Remington, B.C., Westerhout, J., Meima, M.Y., Blom, W.M., Kruizinga, A.G., Wheeler, M.W., Taylor, S.L., Houben, G.F. & Baumert, J.L.** 2020. Updated population minimal eliciting dose-distributions for use in risk assessment of 14 priority food allergens. *Food and Chemical Toxicology*, 139: 111259. https://doi.org/10.1016/j.fct.2020.111259

9.2 SUMMARY AND CONCLUSIONS ON HAZARD CHARACTERIZATION

The amount of data on the characteristics of reactions at ED_{01}/ED_{05} values for different allergens varies considerably in its abundance. Most data are available for peanut, the most extensively studied allergenic food. Reviewing the evidence, the hazard characterization group concluded that data suggested that peanut could be used as an exemplar for other allergens. They also observed that published literature does not contain cases of fatal reactions at less than 5 mg total protein for the priority food allergens examined to date.

The group also reviewed the supporting data from the Remington *et al.* (2020) and Houben *et al.* (2020) manuscripts with a focus on reaction severity characteristics at ED_{01}/ED_{05}/ED_{10}. Over 1 100 data points lent themselves to this analysis. Evidence indicated that reactions at levels of exposure up to and including the ED_{05} could include mild anaphylaxis, but none of the reported reactions met the World Allergy Organization (WAO) definition for severe (i.e. life-threatening or refractory) anaphylaxis (although the group acknowledged that the dataset examined does not preclude the possibility of such a severe reaction). The group also found that, on the basis of the available evidence, the characteristics of objective (externally observable) reactions were no different at ED_{01} and ED_{05}, with up to 5 percent of individuals

with objective symptoms to that level of exposure developing symptoms consistent with anaphylaxis. Furthermore, the expected (very low) rate of severe anaphylaxis would not be expected to differ between ED_{01} and ED_{05}. The group noted that (by definition) around five times more individuals would be expected to develop any objective symptoms to ED_{05} than ED_{01} exposure, and therefore in absolute terms, five times more people would be expected to experience mild anaphylaxis to ED_{05} exposures as to the ED_{01} level. However, given the very significant analytical limitations that currently exist in relation to using ED_{01} rather than ED_{05} as RfDs, the group suggested that the ED_{05} form the basis of the proposed RfDs. It was noted that while fewer individuals would experience allergic symptoms to ED_{01} levels of exposure, the same proportion of reactors would experience anaphylaxis, and use of ED_{01} would not "*minimise the probability of any clinically relevant objective allergic response, to a point where such further refinement meaningfully reduces health impact*", particularly where the "incidental symptoms likely to be elicited in the range of envisioned RfDs are of an acceptable severity". In contrast, use of the ED_{01} would introduce considerable burdens and limitations for monitoring and potential unintended consequences on the application of PAL or other risk management strategies.

In plenary discussion the expert committee endorsed the conclusions on hazard characterization and welcomed the proposal of a single RfD per allergen, rather than a range. Several experts considered that offering a choice of RfDs based on ED_{05} and ED_{01} would be problematic in relation to implementation and would likely lead to confusion over how to decide on one over the other.

9.3 FEASIBILITY OF ALLERGEN GROUPING FOR SIMILAR REFERENCE DOSES (RfD)

Having endorsed the ED_{05}-based RfDs, the expert committee discussed the grouping of RfDs. The rationale for grouping is to simplify implementation of RfDs by having a limited number of values. The concept of grouping is supported by the overlapping confidence intervals for many of the RfDs, as well as the small differences in actual values in several cases. However, several experts did not consider grouping necessary as different allergens have different patterns of use/consumption, which need to be taken into account at the implementation stage. Furthermore, RfDs represent mass amounts of total protein from the allergenic food and need to be converted to concentrations (action levels) for application, bringing into consideration the food intake. In that context, grouping does not seem particularly useful. There was also lack of clarity over the scientific rationale for the process. Finally, grouping may reduce some transparency in the process whereby RfDs have been defined.

Having debated these issues, the expert committee opted for a simplification process. In the first instance, for most allergens, the actual ED05 values on which the RfDs are based were rounded down to a single significant figure on the basis of the size of the confidence intervals. Exceptions were those allergens for which the data were susceptible to a high degree of bias (e.g. cashew, walnut) or where there could be

a high degree of uncertainty about the true value of the ED05 due to the limited number of species tested within a food group (e.g. fish, shrimp/crustacea). Due to these uncertainties, fish and shrimp/crustacea ED05 values were rounded down further than the other foods.

Shrimp ED05 values (280 and 489 mg shrimp protein) were rounded down to the recommended RfD for crustacea of 200mg crustacea (shrimp) protein because the analysis relied on a few species of shrimp to provide data for the group of crustacea and the additional, more conservative rounding was considered appropriate when considering the diversity of crustacea species consumed.

Following the rationale provided by crustacea, the fish ED05 values (12.1 and 15.6 mg fish protein) were rounded down to the recommended RfD of 5mg fish protein (instead of 10mg) because the analysis relied heavily on one species (cod), and the additional, more conservative rounding was considered appropriate when considering the diversity of fish species consumed.

The resulting RfD values were then collated into different ranges and further simplified within the ranges, using the same principle of rounding down. The table below summarises the overall outcome.

Table 17 summarizes the overall outcome.

TABLE 17 **CONSENSUS REFERENCE DOSE (RfD) RECOMMENDATIONS FOR CODEX PRIORITY ALLERGENS**

	RFD RECOMMENDATION (mg total protein from the allergen c source)
WALNUT (AND PECAN*)	1.0
CASHEW (AND PISTACHIO*)	1.0
ALMOND**	1.0
MILK	2.0
PEANUT	2.0
EGG	2.0
SESAME	2.0
HAZELNUT	3.0
WHEAT	5.0
FISH	5.0
CRUSTACEA	200

Source: Authors' own elaboration.
* See considerations below.
** Provisional.

SCIENTIFIC RATIONALE FOR RFD

- The RfD meet the criterion of "exposure without appreciable health risk" This was defined as:
  - a probability of objective symptoms of <5 percent in the population of individuals with a relevant IgE-mediated food allergy when ingesting a dose not exceeding the RfD;
  - in those who do develop objective symptoms to a dose not exceeding the RfD, a probability of non-severe anaphylaxis (according to the World Allergy Organization definition) of <5 percent;
  - a risk of severe anaphylaxis (according to the World Allergy Organization definition) of <1:100 000 person years in the population of individuals with a relevant IgE-mediated food allergy; and
  - a risk of fatal reaction of <1 per million in the population of individuals with a relevant IgE-mediated food allergy when ingesting a dose not exceeding the RfD. Note: No fatal anaphylaxis events have been reported following exposure to a dose not exceeding the RfD.
- When non-severe anaphylaxis was observed in clinical studies, it resolved in at least 80 percent without any treatment, and >98 percent of the remainder of cases responded to first line treatment (epinephrine/adrenaline).

CHAPTER 10
FORMULATION OF RECOMMENDATIONS

After extensive discussion, the expert committee reached a consensus on reference doses (RfD) for priority allergenic foods, meeting the criterion for HBGV that they should "reflect a range of exposure without appreciable health risk" (EHC 240, Chapter 5 [FAO and WHO, 2020b]).

RECOMMENDATION

- The Ad hoc Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens recommends the RfD in the table below for adoption by Codex for the use of risk management of UAP in foods, together with an evaluation period of at least five years:
  - The use of risk management options might include implementation of PAL, monitoring and management of allergens in the supply chain or in production facilities, and management of health hazards of UAP (e.g. recalls), and so forth.
- The difference in the public health impact of choosing a more stringent RfD is expected to be negligible in terms of reducing significant public health risk. A more stringent RfD would introduce considerable burdens and limitations for monitoring and potential unintended consequences on the application of PAL or other risk management strategies. This is particularly pertinent with respect to potential limitations to food choice for individuals with IgE-mediated food allergies.
- The RfD is not appropriate, nor intended to be used to define "allergen-free" labelling.

TABLE 18 **CONSENSUS REFERENCE DOSE (RfD) RECOMMENDATIONS FOR CODEX PRIORITY ALLERGENS**

	RFD RECOMMENDATION (mg total protein from the allergenic source)
WALNUT (AND PECAN*)	1.0
CASHEW (AND PISTACHIO*)	1.0
ALMOND**	1.0
MILK	2.0
PEANUT	2.0
EGG	2.0
SESAME	2.0
HAZELNUT	3.0
WHEAT	5.0
FISH	5.0
CRUSTACEA	200

Source: Authors' own elaboration.
* See considerations below.
** Provisional.

SCIENTIFIC RATIONALE FOR RFD

- The RfD meet the criterion of "*exposure without appreciable health risk*". This was defined as:
  - a probability of objective symptoms of <5 percent in the population of individuals with a relevant IgE-mediated food allergy when ingesting a dose not exceeding the RfD;
  - in those who do develop objective symptoms to a dose not exceeding the RfD, a probability of non-severe anaphylaxis (according to the World Allergy Organization definition) of <5 percent;
  - a risk of severe anaphylaxis (according to the World Allergy Organization definition) of <1:100 000 person years in the population of individuals with a relevant IgE-mediated food allergy; and
  - a risk of fatal reaction of <1 per million in the population of individuals with a relevant IgE-mediated food allergy when ingesting a dose not exceeding the RfD. Note: No fatal anaphylaxis events have been reported following exposure to a dose not exceeding the RfD.
- When non-severe anaphylaxis was observed in clinical studies, at least 80 percent resolve without any treatment, and >98 percent of the remainder respond to first line treatment (epinephrine/adrenaline).

ANALYTICAL CONSIDERATIONS CONSEQUENT ON APPLICATION OF REFERENCE DOSE (RfD)

- RfD can be implemented and monitored to some degree with current analytical capabilities, with the acknowledgement that a number of limitations still exist.
- A conversion of an RfD (mg dose of total protein from the allergenic source) into action levels (mg total protein from the allergenic source/kg of food) based on food consumption/intake information is necessary for adherence to the established RfD:
  - Reporting units need to be expressed in mg total protein from the allergenic source/kg of food.
  - Consumption quantities should be based on a percentile consumption estimate per single eating occasion appropriate to the intended protection level; see full report for more details.
  - See action level table below.
- Current analytical deficiencies and/or inconsistencies, include but are not limited to:
  - lack of appropriate methods that are fit for purpose in identification and quantification of fish and wheat;
  - limitations in quantification of all species of crustacean shellfish;
  - inconsistencies with reporting units (need to be expressed in mg total protein from the allergenic source/kg of food);
  - limited availability of reference materials and absence of reference methods;
  - poor recovery or ability to extract proteins from complex food matrices and validation in a diversity of food matrices;
  - need to develop or adapt sampling plans to facilitate the monitoring of adherence to an established RfD.
- Method performance criteria indicate that the limits of quantification (LoQ) of any method utilized for a specific food should be approximately 3-fold lower than the action level for that food in order to account for real-world performance variability and to assure that the analytical result is truly at or below the action level.
- In case analytical capability is insufficient to monitor action levels in adherence with an RfD, a temporary action level could be set at a higher level (at analytical limit) awaiting improved methods. The RfD can help steer the improvement of methods as these provide the performance criteria needed. The full range of ED values, such as those published in Houben *et al.* 2020, can help to assess the risk of such a temporary higher action level.

OVERARCHING CONSIDERATIONS FOR RISK MANAGERS

- Regions will be responsible for defining which food products lie within each consumption category for that region's population and dietary habits, based on a consumption percentile appropriate to the intended protection level; see full report for more details.
- Insufficient data existed for almond, pecan and pistachio.
  - Due to the known cross-reactivities and co-existent allergies between pistachio and cashew, and pecan and walnut, a placeholder RfD for pecan and pistachio are proposed as below:
    - RfD for pecan of 1.0 mg total protein from the allergenic source
    - RfD for pistachio of 1.0 mg total protein from the allergenic source
  - In view of insufficient information for almond, an RfD is proposed at 1.0 mg total protein from the allergenic source in concordance with the lowest RfD for tree nuts.

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ANNEX 1
STUDIES CONSIDERED FROM POTENCY SUBGROUP REVIEW

Studies identified by the potency subgroup during Part 1 of this Ad hoc Joint FAO/WHO Expert Consultation were considered to contain information to help inform the discussion during Part 2 of this Ad hoc Joint FAO/WHO Expert Consultation. However, it should be noted that studies identified in this Annex were not included in the dose-distribution analysis due to a lack of information or unclear information regarding individual food challenge data with objective symptoms.

TABLE A1 **STUDIES CONSIDERED FROM POTENCY SUBGROUP REVIEW**

FOOD	REFERENCE	Selected or unselected population?	Selected or unselected population? Study funded by? Which clinical trial is this study a part of?	Number of individuals reported?	Food challenge dosing scheme reported (Y/N)?	Symptoms reported? Objective, subjective, unclear/mixed?	Potency comments Median dose? Individual thresholds available?
Cashew	Divaret-Chauveau *et al.*, 2018	Unselected	Unselected	108 (52/56) - unclear if all in analysis	Y	Objective	According to this model, the ED05, ED10 and ED50 and their 95% confidence intervals were, respectively, 0.9 mg (0; 3.5); 2.8 mg (0; 9.7) and 57.3 mg (15.0; 216.2) of cashew nut proteins.
Cashew	Elizur *et al.*, 2019	Selected	Selected for patients with walnut allergy immunotherapy - co-sensitized (Nut CRACKER)	19	N - sup material not accessible	Unclear - protocol in supplementary material and not accessible	Reaction dose - median (IQR) - 100 mg cashew protein (IQR 20-388)
Cashew	Sato *et al.*, 2019	Unselected	Unselected	26	N	Subjective and objective (unclear)	Cumulative dose - median (range) - 1.35 g cashew powder (0.1–10 g) (assumed cashew flower, protein conversion 257 mg cashew protein [19–1 900 mg])
Cow's milk	Calvani *et al.*, 2012	Unselected	Unselected (seemingly): prospective study in 66 children referred to allergy clinic with diagnosis or presumed diagnosis of CMA	14	Y	Objective and subjective	Reactive dose - median - 30 ml [990 mg milk protein]
Cow's milk	Eller, Hansen and Bindslev-Jensen, 2012	Unselected	Unselected population; Odense University Hospital and Region of Southern DK	121	Y	Objective and subjective	ED10: 100.2 (52.7–190.5) mg milk protein
Cow's milk	Keet *et al.*, 2012	Selected	Selected: no Hx persistent asthma, anaphylaxis; reactive to <2.5 g of CM protein	30	Yes	Stopping criteria described: rash, upper or lower respiratory tract symptoms, abdominal pain and vomiting, or signs or symptoms of hypotension	Threshold dose - median - 101 mg milk protein (1–151), 31.1 mg milk protein (1.1–551.1), 81.1 mg milk protein (11.1–1 351) depending on treatment group (SLIT, OITB, OITA)
Cow's milk	Longo *et al.*, 2012	Unselected	Unselected; prospective study of specific tolerance induction (SOTI); excluded patients on anti-allergic meds or ill	245	Yes, but some uncertainty about lowest doses; also open challenge; doses administered at 10 mn intervals	Challenge continued if symptoms subjective or transient, dose repeated if doubtful; stopped after persistent or more severe symptoms	Eliciting dose - median (IQR) - 8 ml (4–16 ml) - converted to 264 mg milk protein (132–528 mg) (tolerated dose reported converted to eliciting dose)
Cow's milk	Rolinck-Werninghaus *et al.*, 2012	Unselected	Unselected population referred to allergy clinic	305	Yes, 3–3 300 mg total fresh CM protein	Subjective and objective	Reactive dose - median of 1 000 mg CM protein - rough estimate of information deduced from graphic data, but grading scheme of reaction could mix subjective with objective
Cow's milk	Sánchez-García *et al.*, 2012	Unselected	Unselected; consecutive patients attending allergy clinic	105	Yes 0.2–145 ml of fresh whole milk (protein content 30 mg/ml)	Objective	Eliciting (reactive) doses – mean – 10 ml (SD 45.7 ml, range 0.7–145 ml) (protein mean 150 mg milk protein [range 21–4 410 mg])
Cow's milk	Vazquez-Ortiz, 2013	Unselected	Unselected, consecutively recruited from allergy clinic; medical exclusions according to guidelines	81	Yes - SEICAP protocol. Doses 1.2 to 145 ml cow's milk (fresh pasteurized containing 30 mg/ml total protein)	Subjective and objective	Threshold dose - median (range) – 5 ml (0.2–150ml) (150 mg [6–4 500] protein)
Cow's milk	Lee *et al.*, 2013	Selected	Subset of wider study of CM allergy up to 16 years. 7–12 month olds with DBPCFC-confirmed CMA selected according to compare specific IgE and IgG4 levels with matched non-CMA 7–12 months old (OFC negative)	31	Yes: 0.5, 1.0, 2.0, 5.0, 10 ml of CM in Neocate at 30 mn intervals	Unclear - stopping criteria: "adverse reaction" or 10 ml CM	Data provided for 2 subgroups: oral desensitization (DBPCFC threshold <5 ml [165 mg milk protein]: 3, = >5 ml [165 mg milk protein]: 11 and elimination diet threshold <5 ml [165 mg milk protein]: 4, = >5 ml [165 mg milk protein] 8)

TABLE A1 **STUDIES CONSIDERED FROM POTENCY SUBGROUP REVIEW** (continued)

FOOD	REFERENCE	Selected or unselected population?	Selected or unselected population? Study funded by? Which clinical trial is this study a part of?	Number of individuals reported?	Food challenge dosing scheme reported (Y/N)?	Symptoms reported? Objective, subjective, unclear/mixed?	Potency comments Median dose? Individual thresholds available?
Cow's milk	Okada, *et al.*, 2015	Selected	Selected for reactions to low-dose of heated cow's milk (<25 ml) and then tested with a very low-dose (3 ml)	32 with previous OFC at 25 ml or less		Objective	Threshold dose - median (range) - 412.5 mg milk protein (99–825 mg) (12.5 ml [3.0–25 ml])
Cow's milk	Wood *et al.*, 2016	Selected	OIT Selected for OIT	Omalizumab = 28, Placebo = 29	Y	Mix of subjective and objective (clear objective and marked subjective, e.g. persistent abdominal pain)	Cumulative eliciting dose - median (IQR) - Omalizumab 100 mg (20–300), placebo 30 mg (20–300) (reported as cumulative successfully consumed dose - median [IQ] Omalizumab 20 mg [0–100 mg], placebo 10 mg [0–100 mg] - CONVERTED TO CUMULATIVE ELICITING DOSE)
Cow's milk	Takahashi *et al.*, 2016	Selected	OIT Prospective longitudinal study of OIT; patients allowed to choose active or placebo group; open challenges	37	Yes - 0.2, 0.5, 1,2, 5. 10, 20 and 40 ml Started at 2 ml if no anaphylaxis	Objective	Median 2 ml (0.1-40) (66 mg total CM protein [3.3–1 320] based on Taylor *et al.* 2014)
Cow's milk	Capucilli *et al.*, 2018	Unselected	Unselected	Total positive milk challenges: 70 BM tolerant, BM avoid, never ingested	Y (dosing with powdered food)	Objective + abdominal pain	No significant differences seen between BM tol, BM avoid and NI groups.
Cow's milk	Inagaki *et al.*, 2018	Unselected	Unselected	43	N	Objective or persistent severe subjective (not OAS)	Mean +/- SD = 545.8 +/- 573.4 mg milk protein
Cow's milk	De Schryver *et al.*, 2019	Selected	OIT but relatively unselected with regards to thresholds for inclusion in study, only individuals tolerating 200 ml CM (8 800 mg CM protein) at SBPCFC were excluded	41	Y	Unclear - nothing reported in methods or results	Cumulative reactive dose - mean (SD) – 1 100 mg CM protein (1 496 mg) (in ml liquid milk 25 ml [34.0]) Note added: very high protein content used in this publication
Cow's milk	Ayats-Vidal *et al.*, 2020	Unselected	Unselected	39	Y	Unclear – detailed as appearance of a "Clinical reaction" and then they mention grading by Bock's criteria	Reactive dose - mean (range) - not specific as to cumulative or discrete – 478 mg milk protein (9.9–2 016 mg) (14.5 ml [0.3–61.1 ml]) *likely cumulative by dosing scheme but not specified
Egg	Santadusit, Atthapaisalsarude and Vichyanond, 2005 (identified by EG)	Unselected	Unselected	6	N	Objective	Range of 1/2 – 1 egg = 3.15–6.3 g egg protein using conversion assumptions
Egg	Eller, Hansen and Bindslev-Jensen, 2012	Unselected	Unselected population; Odense University Hospital and Region of Southern DK	237	Y	Objective and subjective	ED_{10}: 5.36 (3.0–9.6) mg egg protein
Egg	Okamoto *et al.*, 2012	Unselected	Unselected population, but no atopic dermatitis	105	Yes	Open challenges; objective symptoms only	Reactive dose - median (IQR) - 168 mg egg white protein (<84–336 mg) [60 positive to OFC: <0.8 g: 25; 1.6 g: 13; 3.2 g: 11; 6.4 g: 11 (converted to median [IQR] - 1.6 g [<0.8 - 3.2 g] – protein using 10.5% for EW – median [IQR] 168 mg egg white protein [<84–336 mg])

TABLE A1 **STUDIES CONSIDERED FROM POTENCY SUBGROUP REVIEW** (continued)

FOOD	REFERENCE	Selected or unselected population?	Selected or unselected population? Study funded by? Which clinical trial is this study a part of?	Number of individuals reported?	Food challenge dosing scheme reported (Y/N)?	Symptoms reported? Objective, subjective, unclear/mixed?	Potency comments Median dose? Individual thresholds available?
Egg	Rolinck-Werninghaus *et al.*, 2012	Unselected	Unselected population referred to allergy clinic	313	Yes, 5–4 600 mg total pasteurized hen's egg protein	Subjective and objective	Reactive dose - median of 460 mg Egg protein - Rough estimate of information deduced from graphic data, but grading scheme of reaction could mix subjective with objective
Egg	Libbers *et al.*, 2013	Unselected	Retrospective analysis comparing different matrices. Unselected allergy clinic attendees with suspected egg allergy. Prior anaphylaxis NOT excluded	59	Yes: 1.75 mg – 3.50 mg – 14 mg – 70 mg – 350 mg – 1 750 mg	Objective	3 groups – eliciting dose (mg egg protein): 22.8% fat: 438 (20–993); 31.9% fat: 1 369 (436–2 189); 52.7 % fat: 434 (90–1 763) - no statistically significant differences between groups
Egg	Bartnikas *et al.*, 2015	Unselected	Individuals undergoing cooked egg challenges	6 with objective symptoms	Y	Individual symptoms	Cumulative eliciting dose – median (range) – 2 100 mg egg protein (100–1 1600)
Egg	Escudero *et al.*, 2015	Selected	OIT recruitment but otherwise seemingly unselected based on inclusion criteria and not mention of a limiting eliciting dose	CG = 31, OITG = 30	Y	Mix of subjective and objective	Cumulative eliciting dose mg EW protein – median (range) – CG 162 mg (9–3 600); OITG 72 mg (9–1 708)
Egg	Ballmer-Weber *et al.*, 2016	Unselected	Unselected; medical exclusions: prior anaphylaxis to egg, other severe chronic disease	24	7-step: no further description, but could be deduced from doses reported in results	Objective	Eliciting dose 6.7 mg–9 627 mg whole egg powder (cumulative)
Egg	Gradman *et al.*, 2016	Unselected	Unselected, retrospective study	99	Yes: 0.011 g, 0.044 g, 0.250 g, 0.500 g, 1 g, 7.5 g, 40 g,	Objective	Median 4.305 g [1.8; 9.305] (542 mg whole egg protein [223 mg–1 172 mg] – based on Taylor *et al.*, 2014). Data as cited in text by authors for the 99 challenged individuals - differs from the summary tables in other parts of the Gradman *et al.*, 2016 manuscript.
Egg	Sugiura *et al.*, 2016	Unselected	Unselected - excluded if recent anaphylaxis to small exposure	198	Y	Objective (ASCA 5 points or higher) (ASCA = Anaphylaxis scoring Aichi)	Cumulative dose – median (IQR) - 3.7 g boiled egg white (1.7 g–8.8 g) (Protein conversion 388.5 mg egg white protein [178.5–924 mg])
Egg	Capucilli *et al.*, 2018	Unselected	Unselected	Total positive egg challenges: 115. BE tolerant = 53, BE avoid = 30, Never ingested = 32	Y (dosing with powdered food)	Objective + abdominal pain	Eliciting dose - median (range) - BE tol 3.0 g (0.125–15.75 g); BE avoid 0.69g (0.13–10.0 g); NI egg 0.88 g (0.13–13.88 g). BE avoid and NI were not significantly different. (Not clear if food or protein reported)
Egg	Inagaki *et al.*, 2018	Unselected	Unselected	50	N	Objective or persistent severe subjective (not OAS)	Mean +/- SD = 188.7 +/- 170.3 mg egg white protein
Egg	Martin-Munoz *et al.*, 2019a	Selected	Selected for OIT trial but otherwise seemingly high baseline challenge	CG = 25, OIT = 76	Y	Objective + abdominal pain	Threshold dose - median (min–max) – baseline CG 260 mg egg white proteins (20–660 mg); OIT baseline 220 mg egg white protein (110–2 000 mg).
Egg	Martín-Muñoz *et al.*, 2019b	Unselected	OIT but seemingly unselected with regards to thresholds for inclusion in study	A/B/C = 38/38/25	Y	Objective	Threshold dose - median (min-max) - A/B/C: A = 270 mg PEW protein (55–2 200) / B = 270 mg PEW protein (44–2 500) / C = 260 mg PEW protein (22–660)
Hazelnut	Eller, Hansen and Bindslev-Jensen, 2012	Unselected	Unselected population; Odense University Hospital and Region of Southern DK	174	Y	Objective and subjective	ED_{10}: 15.9 (8.9–28.4) mg hazelnut protein

TABLE A1 **STUDIES CONSIDERED FROM POTENCY SUBGROUP REVIEW** (continued)

FOOD	REFERENCE	Selected or unselected population?	Selected or unselected population? Study funded by? Which clinical trial is this study a part of?	Number of individuals reported?	Food challenge dosing scheme reported (Y/N)?	Symptoms reported? Objective, subjective, unclear/mixed?	Potency comments Median dose? Individual thresholds available?
Hazelnut	Dereci, Koca and Akcam, 2016	Unselected	Unselected, epidemiological study of hazelnut allergy, with DBPCFC confirmation of allergy. Exclusions: anaphylaxis in preceding year	8	Yes; 0.1, 0.3, 0.6, 1.5, 2.5, 5, and 15 g of hazelnut.	Objective	Cumulative dose – range – 63 mg–1 570 mg total hazelnut protein (0.4g–10.0g [full results available] – conversion based on protein values from Taylor *et al.*, 2014)
Hazelnut	Elizur *et al.*, 2019	Selected	Selected for patients with walnut allergy immunotherapy – cosensitized (Nut CRACKER)	15	N – sup material not accessible	Unclear – protocol in supplementary material and not accessible	Reactive dose – median (IQR) – 120 mg hazelnut protein (20–540)
Peanut	Eller, Hansen and Bindslev-Jensen, 2012	Unselected	Unselected population; Odense University Hospital and Region of Southern DK	249	Y	Objective and subjective	ED_{10}: 32.9 (23.6–45.9) mg peanut protein (roasted)
Peanut	Savage *et al.*, 2012	Unselected	Unselected except for medical exclusions (persistent asthma, Hx of severe reaction to peanut, etc.)	14	Referenced Niggeman *et al.* (1994) 7 doses building up to a maximum cumulative dose of 1 000 mg	Subjective and objective	Threshold dose - median (range) – 80 mg (30–380 in one group; 10–700 in the other)
Peanut	Fleischer *et al.*, 2013	Selected	Selected as allergic to peanut and objective symptoms to = <2 g peanut powder (cumulative), randomized into equal placebo and verum groups. Medical exclusions including anaphylaxis to peanut – CoFAR study	40	Yes, but not explicit for baseline	Implicitly, subjective and objective for baseline OFCs	Lowest dose at first symptom median (IQR) – 3 mg peanut protein (0.5–35.5), successfully consumed dose 35.5 mg peanut protein (1.75, 98); SLIT group 10.5 mg peanut protein (0.5–146) – peanut flour 6 mg (1–71) SCD: Placebo group median 71 (3.5 – 196); SLIT group median 21 (1–146)
Peanut	Ludman *et al.*, 2013	Unselected	Retrospective analysis; unselected patients with peanut challenge; excluded if Hx recent anaphylaxis	200 (United Kingdom 100; CH 100); 73 positive challenges (United Kingdom 25, CH 48)	Yes - Cumulative dose up 9.2 g in United Kingdom, 4.43 g in CH	Not described, but very likely criteria based on objective Rx	Average protein dose United Kingdom 2.3 g peanut protein (SD 3.3 g); CH 1.5 g peanut protein (SD 1.8 g). 5 Rx to highest dose in United Kingdom, 8 in CH
Peanut	Schneider *et al.*, 2013	Selected	Clinical trial: rush desensitization after treatment with ant-IgE antibody; patients reactive < = 100 mg peanut flour (186 mg cumulative) –- medical exclusions	13	Yes – but needs to be deduced	Objective	Cumulative eliciting doses – median (range) – 35.5 mg peanut protein (0.5-93 mg) – (discrete 50 mg peanut flour [1–100] [discrete]; cumulative 71 mg peanut flour [1–186 mg])
Peanut	Anagnostou *et al.*, 2014	Selected	Randomized clinical trial of immunotherapy; immediate hypersensitivity on peanut ingestion. Medical exclusion: significant chronic illness (other than allergy-related)	99	Yes; 5, 50, 100, 300, 1 000 mg	Objective and subjective	LOAEL – mean (SD) – 147.9mg peanut protein (SD 274.6) mg (control group); 94.6 mg peanut protein (SD 77.4 mg) (active group); NOAEL 41.5 mg peanut Protein (SD 80.1 mg) (control group) 28.4 mg peanut protein (SD 36.8 mg) (active group). No statistically significant differences between control and active groups at baseline
Peanut	Santos *et al.*, 2015	Unselected	Unselected, consecutive patients for evaluation of BAT	124 challenges, 52 positive (eligible)	7 doses: 0.033, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0	Objective	Cumulative threshold – median (range) – 0.1 g peanut protein (0.033–9.35 g)
Peanut	Narisety *et al.*, 2015	Selected	Highly selected for peanut SLIT or OIT	11 active OIT, 10 active SLIT	Y	Unclear (convincing reaction)	Cumulative threshold dose baseline – median (range) – active OIT 21 mg (6–146), active SLIT 21 mg (1–146)
Peanut	Chalmin and Bourrier, 2017	Selected	Selected for OIT, however children with a reactive dose less than 191 mg peanut were not included	31	Y	Objective plus abdominal pain	Cumulative reactive dose - median 485 mg peanut protein – (mean – 1 052 mg peanut protein [4 207 mg peanut])

TABLE A1 **STUDIES CONSIDERED FROM POTENCY SUBGROUP REVIEW** (continued)

FOOD	REFERENCE	Selected or unselected population?	Selected or unselected population? Study funded by? Which clinical trial is this study a part of?	Number of individuals reported?	Food challenge dosing scheme reported (Y/N)?	Symptoms reported? Objective, subjective, unclear/mixed?	Potency comments Median dose? Individual thresholds available?
Peanut	Jones *et al.*, 2017	Selected	Selected (more sensitive selection criteria for DBV immunotherapy)	46	N (reference to other study with details)	Objective	Cumulative reactive dose – median (range) – 144 mg peanut protein (1–1 044) – (cumulative successfully consumed dose – median [range] – 44 mg peanut protein [0–444])
Peanut	Just *et al.*, 2016	Unselected	MIRABEL stufy; MIRABEL design and inclusion of patients Between April 2012 and December 2013, allergists were asked to include consecutive patients with suspected peanut allergy.	199 with positive OFC			Cumulative reactive dose – mean (sd) – 291 (533) mg peanut protein
Peanut	MacGinnitie *et al.*, 2017	Selected	Highly selected, eliciting dose of 50 mg peanut protein (88 mg cumulative) or less for inclusion in study	37	Y	Objective	Cumulative eliciting dose – baseline DBPCFC – median (range): active group n = 29 38 mg peanut protein (1–88), placebo n = 8 88 mg peanut protein (13–88)
Peanut	van Veen *et al.*, 2016	Unselected	Unselected consecutive allergy clinic patients with suspected peanut allergy	33	Yes: 0.84, 1.6, 8, 42, 210, 1 010 mg Vlieg *et al.* (2004) and Flintermann *et al.* (2006)	Objective	ED 0.86 – 1.6 mg in 5 children; 8–42 mg in 14 children; 210–1 010 mg in 14 children
Peanut	Elegbede *et al.*, 2019	Unselected	MIRABEL study; MIRABEL design and inclusion of patients Between April 2012 and December 2013, allergists were asked to include consecutive patients with suspected peanut allergy.	238	No	Objective	Threshold dose (LOAEL) – median (IQR) – 67 mg (16–244); study is about comparison of different statistical models for evaluating EDp values
Peanut	Gomez *et al.*, 2017	Selected	Selected for patients with history of systemic reactions to peach	Group A (allergic to peanut) = 12	Y	Objective	Methods "dose was calculated as cumulative dose inducing the reaction" – Results "TCD, maximum tolerated dose of peanut" – median (IQR) – 3.4 g peanut (0.9–5.6) (Protein conversion ~850 mg peanut protein [225–1 400 mg])
Peanut	Hourihane *et al.*, 2017	Unselected	Unselected, consecutive allergy clinic patients with peanut allergy	378 challenged; 8 positive	Yes: single-dose challenge at ED_{05}	Objective	8 peanut allergic patients (2.1%) out of 378 challenged reacted to 1.5 mg of peanut protein (ED_{05} inferred from dose-distribution modelling); purpose of study was validation of ED_{05}.
Peanut	Reier-Nilsen *et al.*, 2018	Selected	Selected – OIT recruitment population	96	Y The first challenge dose was chosen to 3 mg peanut protein, and the maximal cumulated dose was 6 443 mg, distributed by 7 incremental steps (3, 10, 30, 100, 300, 1 000, 5 000 mg of peanut protein) of 30 minutes interval	Objective	The median (min, max) for reactivity threshold and LOAEL was 35 (1.0, 3943) and 13.0 (0.8, 1610) mg of peanut protein, respectively. The DBPCFC was regarded positive with the occurrence of moderate objective symptoms even if only part of the current dose had been eaten. Cumulated peanut protein (mg) intake at the time of positive DBPCFC was recorded. The LOAEL was recorded post hoc as the lowest, cumulated amount of peanut protein that elicited the first mild, objective adverse reaction during food challenge was observed, according to the modified Bock's criteria.

TABLE A1 **STUDIES CONSIDERED FROM POTENCY SUBGROUP REVIEW** (continued)

FOOD	REFERENCE	Selected or unselected population?	Selected or unselected population? Study funded by? Which clinical trial is this study a part of?	Number of individuals reported?	Food challenge dosing scheme reported (Y/N)?	Symptoms reported? Objective, subjective, unclear/mixed?	Potency comments Median dose? Individual thresholds available?
Peanut	Watson *et al.*, 2017	Selected	Highly selected from patients being recruited and selected for immunotherapy trials, CoFAR cohort	D = 19 / R = 21	Y		Cumulative dose at first objective symptom – median (IQR) – D = 14 mg peanut protein (444 mg), R = 44 mg peanut protein (144 mg). Cumulative challenge stopping dose – median – D & R = 444 mg peanut protein.
Peanut	Inagaki *et al.*, 2018	Unselected	Unselected	14	N	Objective or persistent severe subjective (not OAS)	Mean +/- SD = 676.6 +/- 412.9 mg peanut protein
Peanut	Vickery *et al.*, 2018	Selected	Aimmune AR101 OIT clinical trial. Highly selected, ED of 100 mg peanut protein or less for inclusion in study	492 (4–17)	Y	Objective	Reactive dose – median (IQR) – 30 mg peanut protein (10–100) (tolerated dose reported, converted to reactive dose)
Peanut	Dua *et al.*, 2019	Unselected	Unselected (TRACE peanut study)	100	Y	Objective	Baseline LOAEL – mean (SD) – 304 mg peanut protein (410 mg) – (ED_{01} 3 mg [1.7, 4.8]; ED_{05} 7.6 mg [4.7, 12]; ED_{10} [12.8 mg 8.2, 19.8]; ED_{50} 80.6 mg [57.9, 112])
Peanut	Soller *et al.*, 2019	Selected	Immunotherapy but seemingly included with OFC to normal dosing diagnostic ranges	83 with baseline OFC	N	Mostly objective, but could have pruritis and a few other subjective mixed in	Eliciting dose – median (IQR) – 240 mg peanut protein (80 –880 mg)
Pecan	Elizur *et al.*, 2019	Selected	Selected for patients with walnut allergy immunotherapy – cosensitized (Nut CRACKER)	46	N – sup material not accessible	Unclear – protocol in supplementary material and not accessible	Reaction dose – median (IQR) – 50 mg pecan protein (125–1 250)
Sesame	Salari *et al.*, 2020	Selected	Selected for adult patients with confirmed history of sesame anaphylaxis	20 (16 positive challenges)	Y - Starting dose 0.003 mg sesame protein – Europrevall scheme	Grading scale (I/II/III/IV) used from Ring 1977 originally used for anaphylactoid reactions. The grading scale for severity is so simple that further contact with the authors is necessary to discern between objective and subjective symptoms.	1 individual – grade 1 symptoms to 0.3 mg sesame protein 1 individual – grade 1 symptoms to 13 mg sesame protein 3 individuals grade 1/2/3 symptoms to 33 mg sesame protein 7 individuals (4 with grade 2; 3 with grade 3) to 133 mg sesame protein 4 individuals (2 with grade 1; 1 with grade 2; 1 with grade 4) to 433 mg sesame protein
Walnut	Elizur *et al.*, 2019	Selected	Selected for patients with walnut allergy immunotherapy (Nut CRACKER)	73	N – sup material not accessible	Unclear – protocol in supplementary material and not accessible	55 active immunotherapy treatment baseline OFC – reaction dose – median (IQR) – 210 mg walnut protein (50–465); 18 control 100 mg walnut protein (35–420)
Wheat	Rolinck-Werninghaus *et al.*, 2012	Unselected	Unselected population referred to allergy clinic	88	Yes, 3–2 800 mg total wheat/gluten powder	Subjective and objective	Reactive dose – median of 2 800 mg wheat protein - Rough estimate of information deduced from graphic data, but grading scheme of reaction could mix subjective with objective
Wheat	Okada *et al.*, 2016	Selected	Selected to individuals reacting to equal to or less than 15 g udon noodles (400 mg wheat protein)	57	N	Objective	Median (range) – 214 mg wheat protein (54–400) – (Initial OFC: median 8.0 g udon noodles, range 2.0–15.0)
Wheat	Inagaki *et al.*, 2018	Unselected	Unselected	17	N	Objective or persistent severe subjective (not OAS)	Mean +/- SD = 1115.9 +/- 822.6 mg wheat protein
Wheat	Burman *et al.*, 2019	Selected	Selected for history of severe wheat allergy	13	Y	PRACTALL criteria for pos/neg challenge – open challenge - otherwise unclear	Cumulative eliciting dose – median (range) – 370 mg wheat protein (10–1 600 mg)

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RISK ASSESSMENT OF FOOD ALLERGENS
PART 2: REVIEW AND ESTABLISH THRESHOLD LEVELS IN FOODS FOR THE PRIORITY ALLERGENS

MEETING REPORT

Knowledge of thresholds constitutes a critical requirement to assessing the risk from allergens, as they are a characteristic of the hazard that allergens present to the food-allergic population.

FAO and WHO reconvened the Ad hoc Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens for a second meeting to provide scientific advice on review and establish threshold levels in foods for the priority allergens.

The expert committee concurred that the benchmark dose/probabilistic hazard assessment approach aligned most closely with the requests of the Codex Committees. After extensive discussion, the expert committee reached a consensus on reference doses (RfD) for priority allergenic foods, meeting the criterion for HBGV that they should reflect a range of exposure without appreciable health risk.

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