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Insects in food and feed – allergenicity risk assessment and analytical detection

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Abstract

Insects and insect-based food products have entered in the European market, carrying along issues of safety and the need of establishing a new legal framework. The consumption of massively reared insects can pose chemical and microbiological risks, and insect proteins are likely to represent a hazard for a subpopulation of allergic individuals. All insect-based products are considered 'Novel Food' and fall under EU regulation 2015/2283, according to which a specific application to the European Commission, followed by EFSA scientific evaluation, is needed before the product is put on the market. The recent EU Regulation 2017/893, entered into force on 1 July 2017, allowed a shortlist of seven insect species to be included in the formulation of feeds for aquaculture. Previously, the addition of any insect to any feed for farmed animals was not allowed, due to the risk of prion-derived diseases. The introduction of this new Regulation raises the issue to switch from a classical detection method based on microscopy to a more sophisticated and species-specific method. The overall aims of this EU-FORA project were (i) to set up a new next generation sequencing (NGS)-based molecular method for the identification of insect DNA in feeds for aquaculture; and (ii) to carry out a conceptual work on a probabilistic quantitative risk assessment focused on the allergenicity of yellow mealworm (*Tenebrio molitor*) employed in foods.

Keywords: Insect, Feed, NGS, allergenicity

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1. Introduction

The use of insects as food or feed is nowadays widespread around the globe. Entomophagy, the eating of insects, is practiced in more than hundred countries, with over 2,000 documented edible species (Jongema, 2017). United Nations recommended the practice as a potential solution to the shortage of world food supplies (van Huis et al., 2013). In countries like Thailand or Kenya, where insects are largely consumed, people shifted from collecting them in the wild to developing mass-rearing facilities (Dobermann et al., 2017). In Western societies, where protein still derives largely from domesticated land animals, insects are virtually synonymous to nuisance. The European Society generally refuses to accept edible insects as food, and deliberate human entomophagy is rare in westernised societies. Insects are associated with dirt, fear of contamination and disease, along with a psychological and biased thinking regarding taste, odour and colour (Raheem et al., 2018). The feeling of disgust to entomophagy in the West contributes to the common misconception that entomophagy in the developing world is prompted by starvation and is merely a survival mechanism (van Huis et al., 2013). However, insects provide more or less comparable amounts of nutrients (proteins, fat, minerals and vitamins) as other meat sources. Proteins are the main components of the dry matter, and due to their content of essential amino acids insects have a high nutritional value. Fat is rich in polyunsaturated fatty acids (PUFAs), with a profile similar to that of fish. For reared insects, the nutritional profile is strictly dependent on the type of feeding. Finally, insects are to be preferred over large land animals due to their ecological footprint: rearing of insects consumes less land and water, and their greenhouse emissions are far lower. On the other hand, the safety of consuming massively reared whole insects or processed material needs to be assessed. Biological hazards can be represented by pathogenic bacteria, mycotoxin-producing fungi, parasites, viruses, antimicrobial resistance genes, while chemical hazards are heavy metals and generally all toxic chemical compounds. In October 2015, EFSA published a scientific opinion on a risk profile related to the production and consumption of insects as food and feed, concluding that'for both biological and chemical hazards, the specific production methods, the substrate used, the stage of harvest, the insect species, as well as the methods used for further processing will all have an impact on the possible presence of biological and chemical contaminants in insect food and feed products' (EFSA Scientific Committee, 2015). Therefore, a case-by-case risk assessment (RA) is required before a new product is placed on the market. Another potential threat to consumers is the presence of proteins able to trigger allergic reactions in sensitised individuals. The studies so far carried out suggested that the risk is plausible, and that it is especially higher for crustacean-allergic people, even though also other categories of allergic patients might be involved (Verhoeckx et al., 2014).

Insects can also be added to common animal feeds, and recent experiments showed that feeds formulated with insects are viable alternatives for broilers (Rumpold and Schlüter, 2013; Schiavone et al., 2017). Aquaculture (the farming of aquatic animals) is a fast-growing industry that has to rely on feeds today primarily formulated with fishmeal and fish oil. Even though insects are part of the natural diet of fish, their addition in feeds for aquaculture has been banned in Europe until recently, due to the risk of prion-derived diseases. Regarding the risks related to the presence of prions, in its opinion EFSA concluded that, compared to the occurrence of hazards in currently authorised protein sources of animal origin, the occurrence of hazards in non-processed insects is expected to be equal or lower, as long as the insects are fed on substrates that do not harbour material of ruminant or human (manure) origin (EFSA Scientific Committee, 2015). EU Regulation 2017/893, amending previous regulations, allowed a shortlist of seven insect species (*Hermetia illucens, Musca domestica, Tenebrio molitor, Alphitobius diaperinus, Acheta domesticus, Gryllodes sigillatus* and *Gryllus assimilis*) to be included in the formulation of feeds for aquaculture. Accordingly, a specific method to detect the presence of insects in these feeds and to discriminate between allowed and not allowed species needs to be developed.

2. Description of work programme

2.1. Aims

The fellow was enrolled for this EU-FORA fellowship under the working programme 'Use of novel DNA- and mass spectrometry-based detection methods for the identification of potential allergenic species and food authentication'. The German Federal Institute for Risk Assessment (BfR), the fellow's hosting Institution, is home of the National Reference Laboratory for Animal protein in Feed (NRL-AP), which major task is the development and validation of qualitative and quantitative methods for the detection of animal and plant species in food and feed. The fellow research focused on the setting up

of a new next generation sequencing (NGS)-based molecular method for the identification of insect DNA in feeds for aquaculture.

Another important training objective of the fellowship was to learn how to perform an allergenicity RA. Such types of assessment are usually performed for example on genetically modified organisms (GMOs), but an evaluation of the potential ability to trigger allergic reactions is also required when an application for a novel food is submitted to the European Commission. The European Regulation (EU) 2015/2283, applicable as from 1 January 2018 and replacing Regulation (EC) 258/97, stipulates that all insect-based products are considered as 'Novel Food', due to the lack of a significant history of consumption in the EU before 15 May 1997. A new concept of probabilistic quantitative RA for food allergens, aimed at tackling the issue of allergen thresholds in food, has been proposed (Spanjersberg et al., 2007). Moreover, the fellow carried out a conceptual work on a full quantitative RA focused on the allergenicity of yellow mealworm (*T. molitor*) employed in foods.

2.2. Molecular identification of insects in feeds

The setting up of an NGS-based method to detect and identify the presence of insect DNA in a complex matrix such as fish feed required several steps.

2.2.1. Selection of the target sequence

After a brief review of the existing literature on insect metabarcoding, a specific region on the mitochondrial gene coding for the cytochrome oxidase subunit 1 (COI) was selected. This region has been shown to be suitable for the identification of a wide range of animal taxa, from small insects to fish and birds (Nelson et al., 2007). It is one of the most utilised and effective sequences for metabarcoding and it is also the most sequenced one among the various insect species. Information on the COI sequence of all the seven species of insects allowed by EU Reg. 2017/893 could be retrieved from the Barcode of Life Data System (BOLD), a cloud-based data storage and analysis platform developed at the Centre for Biodiversity Genomics in Canada (www.boldsystems.org). The seven allowed species belong to different orders (Coleoptera, Diptera, Orthoptera), which means they are taxonomically and genetically distant from each other, although some of them are more strictly related (for example *Alphitobius* and *Tenebrio* belong to the same subfamily Tenebrioninae, likewise *Acheta*, *Gryllodes* and *Gryllus* all belong to the Gryllinae subfamily). Taxonomical proximity equals reduced genetic variability, and that is why a hypervariable known region like COI was selected.

The number of COI sequences available for each of the seven species under investigation is also important, because it provides information on the individual genetic variability internal to the single species. Such information is crucial when analysing NGS data, because it can enable to distinguish between an informative and a non-informative single nucleotide polymorphism (SNP). Four out of seven species had an extensive number of COI sequences stored in the BOLD database, while in the other three cases (*Alphitobius diaperinus, Gryllodes sigillatus* and *Gryllus assimilis*) only two informative sequences for each species were available. Based on this information, eight primers, able to amplify differently sized products on the COI sequence, were retrieved from the literature and partially re-designed for the purpose of our investigation. All primers were designed to anneal to rather conserved sites of the COI sequence, in order to amplify all insect species but no other arthropods.

2.2.2. Endpoint PCR to evaluate the performances of the primers

The NRL-AP possesses a large collection of samples of insect DNA that was screened for our investigation. Firstly, all DNAs were checked for their quality and amplificability, testing them in endpoint polymerase chain reaction (PCR) with a universal primer pair written on COI and able to amplify a large product of around 700 base pairs. Degraded or low quality DNA samples were substituted with freshly extracted material. The combination of the eight primers purchased gave rise to five different PCR products, three of which resulted successful in practice. These three products were obtained on the seven species allowed and on other not allowed species from the DNA collection. Moreover, one pair performed well also on DNA isolated from complex food matrices containing insects, and on heavily thermally processed samples.

Two of the three successful primer pairs were selected to be further employed in the setting up of the final NGS protocol.

2.2.3. Cloning and sequencing of insect barcoding regions

Insects, source of the DNA samples forming the NRL-AP collection, were mostly purchased online or from local pet feed shops for reptiles in Berlin. Some of them did not have a certified origin, and the species indication was provided only by the seller. Sequencing of the COI region of these insects was performed for two main reasons: (1) to make sure of their real genus and species, and (2) to collect more information on the individual genetic variability within this gene, especially in the case of those species where little information was available from the public databases (*Alphitobius diaperinus*, *Gryllodes sigillatus* and *Gryllus assimilis*). Ten different DNA samples belonging to four alleged species were amplified using the same primer pair employed in the amplificability tests, amplicons were purified from the agarose gel and ligated into a commercial plasmid vector. Plasmids were used to transform chemically competent cells of *Escherichia coli* that were grown on selective agar plates. Positive clones were cultivated in liquid medium and used to prepare plasmid DNA samples, which were then submitted for sequencing. The results showed that only 4 out of 10 samples were actually matching the expected species, while in all other cases problems of mislabelling or of DNA contamination occurred. Moreover, 16 potentially new SNPs of *Acheta domesticus*, *Gryllodes sigillatus* and *Gryllus assimilis* were identified.

2.2.4. Setting up of the NGS protocol

NGS experiments were carried out in collaboration with BfR Unit 4SZ, the Study Centre for Genome Sequencing and Analysis. Selected primer pairs were adapted for the protocol of the preparation of the 16S Metagenomic Sequencing Library for the Illumina MiSeq System. Anchored primers were purchased and preliminarily tested in endpoint PCR to verify their ability of amplifying DNA from different insect species. Twelve different admixtures of DNA from 3 allowed insect species (*H. illucens, Acheta domesticus, T. molitor*) and from other sources (*Drosophila melanogaster*, fish, crustaceans, soybean and fishmeal) were prepared and used as a template for the creation of two libraries, one for each primer pair. These libraries were subsequently sequenced on the Illumina MiSeq instrument using paired 300 base pair reads. In this protocol, the ends of each read are overlapped to generate high-quality, full-length reads in a single 65-hour run. The MiSeq run output is approximately > 20 million reads and can generate > 100,000 reads per sample.

2.3. *Tenebrio molitor* allergenicity RA

The ingestion of whole insects or of insect-derived products may trigger the development of clinical symptoms typical of allergenic reactions in sensitised individuals. Mealworms (*T. molitor, Alphitobius diaperinus* and *Zophobas morio*) are frequently used as animal feed, they are easily reared, have a high nutritional value and a low content of chitin, due to the fact that the insect is collected and processed during the larval stage, before developing the exoskeleton. Several rearing facilities can be found nowadays also in Europe, particularly in the Netherlands.

The first step of RA is the hazard identification (Figure 1). Larvae of the yellow mealworm *T. molitor* displayed the ability of provoking allergic reactions both via inhalation or skin contact (Bernstein, 1983) and via ingestion (Freye, 1996). A recent study showed how individuals can become primarily sensitised to mealworm via contact skin or inhalation, produce specific antibodies (IgEs) against mealworm allergens, and report allergic symptoms after ingestion in a clinical controlled oral food challenge (Broekman et al., 2015).

It is also the only insect so far for which a double-blind, placebo-controlled food challenge (DBPCFC) trial was carried out in clinically allergic patients (Broekman et al., 2016). DBPCFC data are the only ones that can be used to define threshold doses for foods eliciting allergic reactions in susceptible patients. The threshold dose is defined to be one that elicits allergic reactions in a given proportion of susceptible patients (Bindslev-Jensen et al., 2002). The definition of these eliciting doses (EDs) characterises the hazard posed by the food allergen, and is the second step of the probabilistic RA protocol proposed by Spanjersberg et al. (2007). In this type of RA, EDs are compared to several input variables, like the chance that an allergic person consumes a certain product, the amount of the consumed product, or the chance that the food contains allergens (and in which concentration), forming together the 'allergen intake' variable. In this way, the probability of occurrence of an allergic reaction can be calculated (Figure 2). Threshold doses of mealworm proteins able to elicit an allergic reaction in 5, 10 and 50% of the population (ED_5, ED_{10} and ED_{50}) were calculated on a very small group (15 individuals) of shrimp-allergic patients, due to the lack of clinically verified mealworm-allergic

patients (Broekman et al., 2016). Such levels are therefore to be referred to a subpopulation of crustacean allergic individuals consuming foods containing processed mealworms. In order to perform a quantitative probabilistic RA of the allergenicity of yellow mealworm, these EDs need to be treated as distributions and compared with exposure data.

For the third step of the RA (intake assessment), data regarding the number of (potential) allergens, the total amount of proteins and the ability of the allergens to survive different kinds of food processing (allergen stability) were collected from the scientific literature. Moreover, based on the currently available information, the main subgroups of sensitised patients at risk of developing a reaction after the ingestion of mealworm-containing foods were highlighted, and estimates of their prevalence within the general healthy population were retrieved. These are all individuals previously sensitised to allergenic sources (cockroaches, crustaceans, house dust mites (HDM), molluscs, nematodes) that hold the power to cross-react with mealworms proteins, due to their shared three-dimensional structure. Cross-reactivity is the only reason for an allergic reaction that can be used in the quantitative RA of a novel food, where estimates of allergy prevalence are known. However, allergy may also occur through 'de novo' sensitisation of previously healthy individuals, but in this case it is impossible to predict how many unsensitised individuals will become primarily sensitised, because the mechanisms and the reasons of allergy are still largely unknown. Finally, in order to complete the exposure assessment, information about the quantity of the allergenic food within a complex food matrix, as well as estimates of the serving size and prevalence of consumption should be included. All these information are unfortunately unavailable at the moment, since insect-based products placed on the market before 2018 are temporarily allowed only in some of the Member States. These products still represent a small niche of the market, but based on the current applications under evaluation by EFSA (general abstracts can be consulted freely on the EFSA webpage, https://ec.europa.eu/food/safety/novel_food/authorisations/summary-applications-and-notifications_en), it is likely that their diffusion will grow among the European consumers in the next years, even though it is hard to predict at which pace.

For the evaluation of allergenicity, risk characterisation, the last step of RA, can have two major purposes: (1) the characterisation of a risk associated with a defined (range of) level(s) of allergen(s) in a food product and (2) the establishment of (safe) limit levels for allergens in food. Risk characterisation needs to be attuned to the ultimate purpose of the assessment, which needs to be specified at the problem formulation stage (Crevel et al., 2014). The allergenicity RA of yellow mealworm is an activity still ongoing, and it will probably take into consideration potential scenarios to cope with the lack of exposure data. The model will be developed in collaboration with BfR Unit 33, Epidemiology, statistics and exposure modelling.

3. Conclusions

Before EU Regulation 2017/893 entered into force (1 July 2017), feeds could be inspected for the presence of insect contamination by using simple microscopy, looking for small insect body parts like wings or legs. Since the allowance of seven species for their productions, detection methods can no longer inform just about the presence of unidentified insects, but they must as well be able to identify these species. Given that thousands of insect species exist but only seven are allowed for inclusion in feeds, the method needs the power to distinguish between genetically distant but also very close species (for example between two species of *Gryllus*, one allowed and one not allowed). Most importantly, the method needs to be open, because in the future more insects might be added to the current list. This shortlist was in fact drawn up by the European Commission based on the existing scientific evidences on the insects currently reared in EU, and it is easy to predict that more species will be introduced in the near future. Molecular investigations using DNA guarantee enough specificity for species identification, but classical endpoint or real-time PCR have the limitation to be closed protocols, which need to be re-established and re-validated every time a new species is added to the existing list. NGS technology has been established already since several years, and applications in food and feed authenticity testing are increasing. The main advantage of this technique is that it can provide genetic information on whatever organism is present within the analysed sample, without the need of a predefined target (untargeted analysis), making it theoretically able to detect any unexpected contamination. However, PCR-free procedures like whole genome sequencing (WGS) require a large amount of high quality starting material, which is difficult to obtain from processed foods or feeds in most cases. Conversely, DNA metabarcoding performed exploiting the high-throughput capacity of NGS platforms is a powerful tool for characterising the biodiversity, and it relies on the preparation of libraries of amplicons targeting specific conserved regions, in which DNA or RNA

fragments are coupled to adapters to allow PCR amplification and sequencing. The identification of the obtained sequence relies on the existence of a large updated database, through which sequences can be compared. Global efforts at storing and sharing DNA sequence data have been underway for several decades, and today GenBank, managed by the United States National Center for Biotechnology Information (NCBI), together with other curated, application-specific DNA sequence databases hosted by other organisations (e.g. Greengenes, BOLD or the JRC GMO-Amplicons database), hold several terabases of nucleic acid sequences completely free for consultation (Haynes et al., 2019). The availability of a sound number of reference sequences is of utmost importance for this kind of analysis, and its lack could represent a major drawback.

The protocol proposed by the NRL-AP is based on the NGS of a small region included in the COI gene, a mitochondrial gene well known to be able to discriminate animals at the species level, which has been already tested successfully on insects (Nelson et al., 2007). The protocol still needs to be tested on model feeds prepared at known concentrations of insects, and subsequently validated on commercial feed samples. At the moment, the EU Reg. 2017/893 does not indicate a maximum tolerated amount of prohibited insect material, which could be translated into a zero tolerance policy. However, the presence of several insects in the facilities where feeds are prepared is common, and it is likely to expect that they can fall into the final product and contaminate it. NGS is a powerful and sensitive technology, and the chance that DNA coming from these unintentionally added insects is indeed amplified and sequenced is not negligible. This might represent an issue for the application of the method in official investigations.

Allergenicity RA is an activity so far considered marginal and included within the broadest safety assessment of GMOs. Its primary goal is to prevent the transfer of an existing allergen or celiac-inducing protein into a new food source and to protect those who are allergic or have celiac disease (Goodman and Tetteh, 2011). This RA has been developed within the past two decades and is currently based on a weight of evidence (WoE) approach, an integrated, stepwise, case-by-case approach that relies upon various criteria used in combination, since no single criterion is sufficiently predictive on either allergenicity or non-allergenicity (Codex Alimentarius Commission, 2003). It includes several steps, but the main idea is that the applicant needs to prove that its product is highly unlikely to contain a new potentially allergenic protein based on its primary amino acid sequence. Currently the same strategy is applied for the evaluation of potential allergenicity of novel foods, but several authors have questioned it, claiming that it is not adequate, that there are limitations to be acknowledged and gaps to be filled (Mazzucchelli et al., 2018). From one side, the protocol is too conservative, because linear sequence similarity does not always lead to *in vitro* cross-reactivity, and even that does not automatically indicate clinical cross-reactivity. From another side, however, the protocol is based on a comparison with known allergens, and it has a limited applicability for novel proteins lacking homology to already identified proteins, disregarding the so-called'de novo' sensitisation. Recently, the group led by Verhoeckx proposed a conceptual strategy for novel foods that combines the WoE for food derived from GM plants and other strategies previously published in the literature. The main point raised was the need to introduce *in vitro* functional IgE testing, such as basophil activation test (BAT) and skin prick tests (SPT), and *in vivo* food challenges (DBPCFC) to verify clinical reactivity in sensitised patients. Also, an accurate preliminary collection of information on the history of the food is advised, in order to assess its potential ability to 'de novo' sensitise new individuals (Verhoeckx et al., 2016).

Whether the WoE approach currently applied by EFSA is used, or a more comprehensive strategy is chosen to assess potential allergenicity, the final outcome of this RA will anyway be deterministic and qualitative. Without available data on a threshold dose for a specific food, it is neither possible to conduct RA nor to focus quality control on efforts which bring the greatest benefit to the allergic consumer. In September 1999, 12 clinical allergists and other interested parties were invited to participate in a roundtable conference to share existing data on threshold doses and to discuss clinical approaches that would have allowed the acquisition of that information. The participants concluded that'thresholds for common allergenic foods are finite, measurable and above zero. However, attempting to reach consensus [...] on the basis of the existing data would probably be premature' (Taylor et al., 2002). A similar conclusion was reached by EFSA few years later (EFSA, 2004). Fifteen years later the situation has not changed much, and scientists still struggle in the identification of precise amounts of allergenic protein, the intake of which will have no effects on 100% of the allergic population. In the same years, some groups tried to propose alternative methods to estimate a threshold dose for foods eliciting allergic reactions in susceptible patients. Bindslev-Jensen and colleagues proposed to use clinical food challenge data coming from *in vivo* human studies to model a

statistical distribution of minimum EDs in the allergic population, introducing for the first time an element of hazard characterisation (Bindslev-Jensen et al., 2002). This bottom-up protocol is borrowed by classical chemical and microbiological risk assessment, and has elements of both approaches. Hazard characterisation for allergens relies on human data from DBPCFC studies, so no animal to man extrapolation of the results is needed. The concept of predicted population EDs is used, where EDp refers to the dose of allergen that is predicted to produce a response in p% of the allergic population. Uncertainty still exists regarding the lowest amount to which sufferers will react, the proportion of sufferers reacting to a defined dose, as well as the relationship between dose and severity for any given individual. However, by applying the probabilistic modelling, uncertainty and variability associated with each input variable are included in the model, therefore there's no need to apply, often arbitrary, uncertainty factors to the risk assessment output (Crevel et al., 2014).

Few years later, starting from this concept of hazard characterisation, a group from TNO (the Netherlands organisation for applied scientific research) developed a probabilistic quantitative risk assessment to predict the likelihood of an allergic reaction, resulting in a quantitative assessment of the risk associated with unintended exposure to food allergens. The intent was to overcome the conservative approach based on worst-case values, generally overestimating the actual risk, by proposing a quantitative RA that put together data collected from controlled food challenges and expressing a'safe' dose distribution, and epidemiological data collected from sampling and measuring the quantity of the allergen source within a certain food or group of foods. A case study on hazelnut proteins in chocolate spread was presented as a proof of concept (Spanjersberg et al., 2007). Since this publication, the same approach has been replicated to assess other food matrices, such as peanut in chocolate tablets (Rimbaud et al., 2010), gluten traces in gluten-free wheat substitutes (Gibert et al., 2013) and recently peanut proteins in highly refined vegetable oil (Blom et al., 2017).

The conceptual work on a full quantitative RA of the allergenicity of yellow mealworm (*T. molitor*) tries to apply the concepts of the stochastic RA to a novel food matrix with a postulated allergenicity power. The main limitation is represented by the scarcity of data, both for the hazard characterisation and for the exposure assessment. To overcome these limitations, the model tries to use all retrievable information, either numerical or non-numerical, and needs to run scenarios that take into account the uncertainty.

The EU-FORA Project consists in a practical programme addressed at early- to mid-career scientists working in food safety organisations across Europe, which aims to increase the expertise and capacity available to risk assessment bodies at both the European and national levels (Bronzwaer et al., 2016). Selected candidates are fully integrated into the work of the hosting site, and thanks also to the training modules spread during the year, they develop their knowledge and experience in food risk assessment. BfR actively contributed to the programme since its early development stage, and it provided a suitable training environment. The presence of highly qualified personnel with different scientific backgrounds has encouraged the fellow to widen the spectrum of the investigation and propose collaborations with two other units, specifically the Study Centre for Genome Sequencing and Analysis (Unit 4SZ) and the Epidemiology, statistics and exposure modelling unit. Overall, the fellow acquired the tools and the ability to carry out an independent qualitative risk assessment, and received the necessary training to understand and collaborate to a full quantitative stochastic risk assessment, regardless on the topic. Particularly, the aspect of allergenicity food assessment has been deepened over the all year, following as well the natural inclination that derived from the personal scientific background of the fellow.

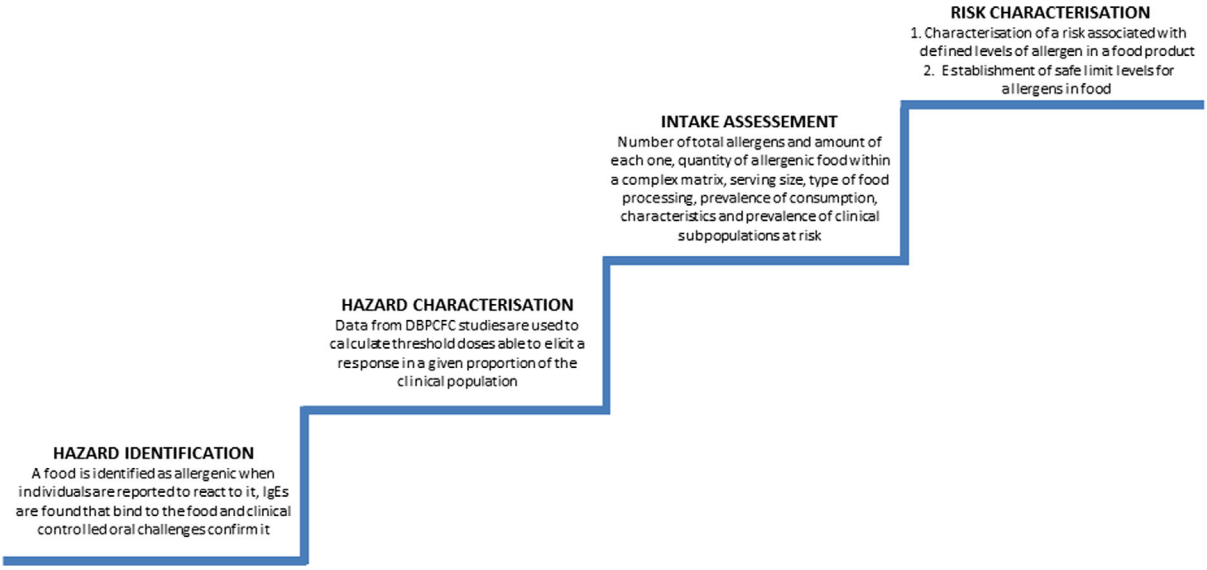

Figure 1: Steps of allergenicity risk assessment

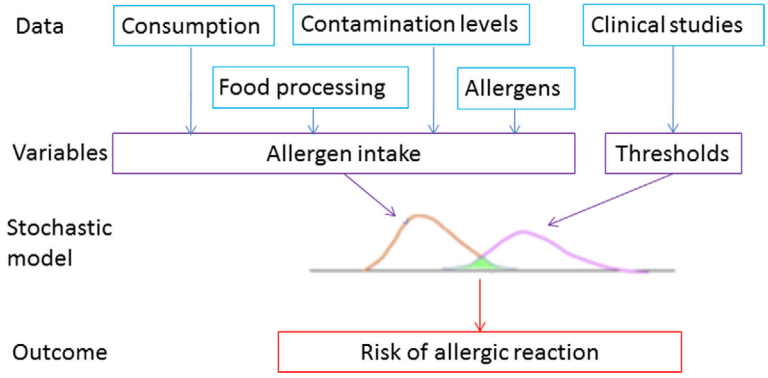

Figure 2: Probabilistic risk assessment for food allergens

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Abbreviations

BAT	basophil activation test
BfR	Bundesinstitut für Risikobewertung
BOLD	Barcode of Life Data System
COI	cytochrome oxidase subunit 1
DBPCFC	double-blind placebo-controlled food challenge
ED	eliciting dose
GMO	genetically modified organism
HDM	house dust mites
IgE	immunoglobulin E
NCBI	National Center for Biotechnology Information
NGS	next generation sequencing
NRL-AP	National reference laboratory for animal protein
PCR	polymerase chain reaction
PUFAs	polyunsaturated fatty acids

RA	risk assessment
SNP	single nucleotide polymorphism
SPT	skin prick test
TNO	the Netherlands organisation for applied scientific research
WGS	whole genome sequencing
WoE	weight of evidence