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REVIEW ARTICLE

Respiratory allergenic potential of plant-derived proteins: Understanding the relationship between exposure and potency for risk assessments

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Abstract

Botanical ingredients (ingredients derived from plants) are finding increasing application in personal care products and the public perceives these ingredients to be safe. However, some proteins in botanicals have the potential to cause immediate-type (IgE-mediated) respiratory allergic reactions. Although reports of such reactions are uncommon, when they do occur, they can be severe. Experience with soap containing wheat proteins illustrated that under certain specific conditions, consumers may be affected. Establishing safe exposure levels for botanical proteins has been challenging. Industrial enzymes provide a rich reference dataset based on their historical association with allergic reactions among workers, which includes robust dose–response information. In the absence of similar data on the potency of plant proteins, a conservative default approach has historically been applied based on information derived from allergenic enzymes. In this article we review the historical default approach and dataset for setting limits for plant proteins in botanical ingredients based on analogy to industrial enzymes followed by a synthesis of literature data on allergic reactions following inhalation exposure to plant-derived proteins. The aim is to share relevant background information and display the relationship between exposure and potency as a first step in the development of a strategy for the development of an improved approach to support the risk assessment of plant-derived proteins.

Keywords

botanicals, consumer products, occupational exposure levels, protein allergen, respiratory sensitization type 1 allergy, risk assessment

History

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Background

The need for better risk assessment methods

Consumer demand for products containing plant-derived extracts (botanicals) has grown significantly over recent years, due in part to the belief that they are natural and therefore safe (Nohynek et al. 2010, Antignac et al. 2011). However, natural does not necessarily equate with safe, as plant-derived natural ingredients have been linked to a significant number of recorded cases of adverse health effects (Nohynek et al. 2010; Antignac et al. 2011). Importantly, proteins found in plant-derived extracts can be associated with IgE antibody-mediated (otherwise known as immediate-type hypersensitivity or type-I allergy) responses (Codreanu et al. 2006; Pecquet et al. 2002; Seitz and Trautmann 2011; Teshima 2014; Pootongkam and Nedorost 2013; Vansina et al. 2010). IgE antibody-mediated immunological responses are responsible for the vast majority of allergic responses described for proteins (Heederick et al. 2002). Improved methods to assess the safety of plant proteins in consumer products for the respiratory allergy endpoint are needed given that the demand for natural products is expected to continue, and existing methods are founded on analogy to occupational enzymes that, while presumed to be protective, treat all proteins as equally potent.

Definitions

For the purposes of this article it is useful to provide some working definitions of terms relating to allergy and allergic disease. These are provided below, but it is important to acknowledge that these are not the only, and not necessarily the best, definitions, but are believed to be appropriate for the issues addressed in this article.

Allergy: the adverse health effects that may be associated with the stimulation of a specific immune response. Allergy develops in 2 phases. In the first phase (induction phase) exposure to an allergen results in immunological priming and the development heightened responsiveness (sensitization). In the second phase (elicitation phase) the sensitized subject is exposed again to the inducing allergen and mounts an accelerated and more aggressive secondary immune response resulting in inflammation and clinical symptoms of an allergic reaction.

Sensitization: a state of heightened responsiveness to a specific allergen due to immunological priming

Allergic reaction: a reaction elicited in a sensitized subject following second or subsequent exposure to the inducing allergen

IgE antibody-mediated allergy (also known as immediate-type hypersensitivity or type-I hypersensitivity): allergy associated with the induction of an IgE antibody response. This class of antibody has the ability to bind to the membrane of mast cells and basophils, and this is a key event during the acquisition of sensitization. Following subsequent exposure to the same allergen, IgE antibody bound to mast cells or basophils will be cross-linked and this in turn leads to cellular activation and degranulation, and the release of inflammatory mediators that trigger an allergic reaction

Atopy: A predisposition to mount IgE antibody-mediated allergic responses

General considerations and challenges

The responses to protein constituents of topically applied products are primarily described in the literature in the context of the local effects observed. The most frequently described responses are urticaria, rhinoconjunctivitis, and angioedema, although severe systemic responses including asthma and anaphylaxis have also been reported. Interestingly, one of the most significant consumer issues with a plant-based protein-containing product that has been widely reported is that related to the use of hydrolyzed wheat in bar soap in Japan. In 2009, two patients were identified who experienced exercise-induced anaphylaxis that was determined to be associated with use of a bar soap containing a hydrolyzed wheat protein of relatively small size (30–50 kD average). By June of 2013, 1900 cases had been reported (Teshima 2014). The majority of these patients suffered angioedema, particularly around the eyes; many developed symptoms after ingesting normal wheat proteins, but were not sensitive to wheat prior to using the soap and exercise-induced anaphylaxis developed in a significant subset. The total number of cases has been updated recently to 2100 and the concentration of wheat protein in the soap described (0.3%) (CIR 2014). It is suggested that both dermal

and mucous membrane exposures are important (Chinuki and Morita 2012), but the possibility of an inhalation exposure component has not been considered.

It has been hypothesized that damaged skin, including that resulting from atopic dermatitis or physical damage, may facilitate protein penetration; however, dermal exposure to proteins via intact skin has generally not been considered to be associated with the induction of sensitization (Pease et al. 2005; Smith Pease et al. 2002; Troyano et al. 2011). In addition, the potential for respiratory exposure in the etiology of these cases has almost never been considered. As summarized by Troyano et al. (2011), prospective clinical studies designed to assess the safety of prototype enzyme containing personal care products such as a bar soap and a body lotion revealed that there can be significant inhalation exposure to these types of products. For the bar soap, laboratory measurements showed that the soap aerosolized in the shower with an average exposure to enzyme of 10.9 ng/m³ and that 6% test subjects developed IgE antibody to the enzyme after 4–6 months of use of the bar soap. For the body lotion, measured levels aerosolized in the shower were 0.25 ng/m³ to 0.50 ng/m³ and specific IgE antibody was demonstrated in 0.46% of subjects, 1 after 6 months of product use, 2 after 15 months of product, and a fourth 3 months after study termination. None showed symptoms associated with product use (Sarlo et al. 2004). While the authors hypothesized that other exposures such as direct mucosal contact and inhalation of enzyme on shed skin cells may also have played a role in the sensitization process, these data clearly demonstrate that there was direct inhalation exposure to aerosolized enzyme associated with normal product use scenarios.

As mentioned previously, the relevance of skin exposure in the acquisition of respiratory sensitization to protein allergens is an area of potential importance, but remains somewhat controversial. The argument is that skin exposure to a protein allergen, at sufficient concentrations, may have the potential to induce an allergic response and the production of IgE antibody resulting in systemic sensitization, including sensitization of the respiratory tract. Furthermore, the view that exposure only occurs through damaged or abraded skin is being updated, with a growing appreciation that exposure to proteins via intact skin sites can also result in the elicitation of an immune or allergic response (Kimber et al. 2014). The assumption is that in many instances proteins encountered at skin sites can gain access to the viable epidermis and engage with cutaneous dendritic cells (DCs), which will in turn initiate the development of an adaptive immune response (Kimber et al. 2014). Moreover, the ability of proteins encountered on the skin to elicit immune/allergic responses is consistent with descriptions of protein-induced allergic contact dermatitis and contact urticaria (Amaro and Goossens 2008). In theory, it is possible for sensitization of the respiratory tract to be acquired through skin contact with a protein allergen. However, current assessment paradigms relate almost exclusively to inhalation exposures and are inadequate to define the relative importance of non-respiratory tract exposures. Thus, occupational exposure guidelines have focused primarily on opportunities for inhalation exposure (Heederik et al. 2012; Nielsen et al. 2012). Heederik et al. (2012) reviewed the literature on reduction of occupational asthma via exposure reduction and skin and respiratory protection. They noted that the role of skin

exposure in the induction of occupational asthma (including from high molecular weight [MW]) allergens has been raised as a concern based on sensitization via the skin in animal models, case reports, clinical experience, and limited epidemiological evidence. The only studies they cite of relevance to the relative role of skin versus inhalation exposure concern isocyanates, a family of potent low-MW respiratory sensitizing chemicals. However, such data are not relevant for consideration of the acquisition of respiratory sensitization to protein allergens.

This review will focus primarily on inhalation exposure. This is partly a pragmatic decision based upon the difficulty of estimating accurately the relative contribution that may be made by protein exposure via the skin and consistent with the expectation that in mixed exposure scenarios inhalation will play the predominant role in driving sensitization of the respiratory tract to proteins. This approach is supported by the post-market data that indicate that the limits set on the basis of the current paradigm (inhalation exposure) are protective for all combined routes of exposure.

A further major challenge in establishing acceptable exposure limits for safety risk assessment of plant-derived proteins are the facts that there is little information on the potency of plant proteins for the induction of IgE antibody-mediated responses in humans and a lack of well-accepted methods to prospectively determine the relative potency of protein allergens (other than enzymes). The guinea pig intra-tracheal test and subsequently the mouse intra-nasal test have been used effectively to rank enzyme potency for establishing occupational exposure guidelines (OEG) for the enzyme chemical domain. However, these tests were never evaluated thoroughly for non-enzyme proteins or for non-occupational exposures. Moreover, they no longer are acceptable for evaluating ingredients in cosmetic products as a result of the animal testing ban in the European Union, even if they could be adapted successfully. Kimber and Basketter (2014) have recently elaborated on the challenges of adapting these tests to the wider domain of non-enzyme proteins.

Approach for assessing respiratory allergy risk of proteins

A default strategy used for establishing limits for the protein content of botanical ingredients relies on previous experience with laundry enzymes and links safe limits defined for enzymes to projected inhalation exposures from various product types resulting in presumed highly conservative, but very restrictive, botanical protein concentration limits (Troyano et al. 2011). Post-market evaluations of consumer comments for P&G's protein-containing products support that this approach has been adequately protective. Respiratory allergy effect reports are sufficiently distinctive that it is possible to detect even a low number of reports as demonstrated by the historical experience with Crotein Q (derivatized, hydrolyzed collagen) (Troyano et al. 2011). Importantly, use of this ingredient pre-dated institution of procedures to evaluate the MW distributions of protein hydrolysates and a default approach to consider any hydrolysate fraction proteins > 2.5 kDa to be potentially allergenic (Troyano et al. 2011). The end result of this series of conservative assumptions is an extremely low protein content limit that, based on post-market data, appears

to be protective for all combined routes of exposure. If methods are developed to rank potency and support a relaxation of the strict limits for some proteins it will be important to revisit the potential role of dermal exposure.

A major confounding factor in the safety assessment of botanical ingredients is that most ingredients of interest for personal care products are not purified plant proteins, but crude mixtures containing a large number of proteins. In the absence of methods to characterize the relative allergenic potential of protein components in the mixtures, the default approach judges the entire protein content as potentially highly allergenic. Based on analogy to food protein allergens it can be hypothesized that only a small subset of the proteins contained in many of these extracts is potentially allergenic in nature. In the absence of methods to establish protein-specific guidelines, the exposure guideline for consumer exposure to protein-containing botanical ingredients has been based on prospective clinical studies using personal care products formulated with protease. In these studies, exposure to aerosolized protease at levels > 0.1 ng/m³ resulted in positive skin tests to the enzyme in a small percentage of subjects (Sarlo et al. 2004). These individuals were positive for specific IgE to protease, and thus were confirmed to be sensitized, but did not necessarily display clinical symptoms on challenge. These clinical data and associated measured protein concentrations from aerosolized protein during showering for either direct-use product types (soap, shower gel, shampoo, and conditioner) or indirect exposures (body lotion) were used to establish benchmark aerosolized protein concentrations. Inhalation exposure to protein for proposed new uses of botanical ingredients in personal care products is estimated by linear extrapolation from this historical clinical data set based on product total protein concentration and amount of product used (from consumer habits and practices data). The Japanese wheat soap experience is another useful point of comparison (beyond the P&G post-marketing data). This soap was primarily marketed as a feminine face wash product. Direct data are lacking on aerosolized protein following use of face wash products at the sink, so the presumed worst case based on the showering model has been assumed in order to calculate an approximate exposure estimate. The wheat-containing facial bar associated with wheat sensitization in Japan was reportedly formulated with 0.3% protein (CIR 2014). The default approach based on shower aerosolization and scaling based on the amount of bar soap product used for facial cleansing results in an estimated airborne protein concentration of ~1 ng/m³ or an order of magnitude higher than the internal exposure benchmark for this model. This suggests that inhalation exposure along with dermal and mucous membrane exposure could have played a role in sensitization.

Historically, the default approach separated IgE antibody-mediated responses to ingested food proteins from those responses induced by inhalation, as if the potentially important allergens for these two routes of exposure represent distinct populations. Recent evidence suggests that this may be an artificial boundary. Cartier (2010) summarized information on inhalation exposure to food allergens and occupational asthma, and presented a list of foods implicated in occupational protein allergy. This author notes that exposure can occur as a result of inhalation of dust and aerosolized proteins generated

during physical food processing or cooking and that it is difficult to separate the role of exposure concentration compared with intrinsic allergen potency in determining the prevalence rates for allergic asthma across different food proteins without air concentration monitoring information. Ramirez and Bahna (2009) note that the most commonly reported food allergens via the inhalation route are wheat, crustaceans, soy, peanut, hen's egg, and cow's milk. The "big 8" food allergens accounting for more than 90% of the food allergic reactions are wheat, crustacean shellfish, fish, soybeans, peanuts, eggs, cow's milk, and tree nuts (US FDA). While fish and tree nuts have not been reported to be significant occupational allergens, there are reports of sensitization following exposure by non-oral routes (inhalation/skin) to both of these foodstuffs (Asero and Antonicelli 2009). Ladics et al. (2014) note that conformational epitopes are commonly recognized for inhaled allergens while food allergens have generally been characterized based on linear epitope sequences. They add that conformational epitopes may also be important for food allergens if they survive the digestive process. This conversely suggests that food proteins may have the potential to induce allergy via inhalation via both the same epitopes that trigger food allergy as well as by epitopes that are destroyed in the digestive process. Troyano et al. (2011) noted that the Food Allergy Anaphylaxis Network (FAAN) advises consumers with food allergies to read cosmetic labels and to avoid eliciting reactions to materials of concern. Thus, formulating products with major food allergens should be avoided to prevent eliciting symptoms in already allergic individuals. However, a risk not discussed is inducing *de novo* allergies to food allergens by inhalation, mucosal, or dermal routes of exposure. This apparently occurred on a relatively broad scale with hydrolyzed wheat (discussed above), but there are many case studies on breaking oral tolerance as a result of inhalation or dermal exposure to foods (plant and animal based). It has also been suggested that exposure via the skin or inhalation to novel food proteins for which oral tolerance has not yet developed due to a lack of oral exposure may result in an allergic response when the food is ingested later (Asero and Antonicelli 2009; Ramirez and Bahna 2009).

In conclusion, it seems that the distinction between proteins that are of concern as potential allergens following oral ingestion and those that are of concern for inducing allergy following inhalation exposure is an artificial distinction. While clearly there may be route-dependent differences in response there appears to be a substantial overlap in allergens of significance across routes.

In the absence of validated test methods for ranking the respiratory allergy potency of botanical proteins, an alternative approach is evaluation in the context of human occupational exposure/effect data for reference proteins. It is acknowledged that this approach is not without challenges, particularly due to the inexact estimations of occupational exposures, the potential for mixed inhalation and skin exposures, exposure to mixtures of potentially allergenic agents in the workplace, and the challenges of clearly differentiating allergic from irritant responses in the context of the published data. However, the proposal explored in this review is that the data are sufficient to group allergens/mixtures into relative potency bins. Thus, the available information on respiratory allergy responses in humans to plant-derived proteins via the inhalation route is

summarized and attempts are made to semi-quantitatively estimate associated protein/allergen exposures. Although this review is focused on plant products, it is of note that the inhalation of proteins from sources other than plants has been implicated as causing respiratory allergy, with the most well-documented examples being hens' egg in egg-processing plants or in the baking and confectionery industries and crustacean proteins in the commercial preparation of shellfish (Baur and Bakehe 2014; Bernstein et al. 1987; Escudero et al. 2003; Gautrin et al. 2010; James and Crespo 2007).

Occupational exposure to protein respiratory allergens and occupational exposure levels

A detailed literature search was conducted using Toxline, Medline, and Scopus®. A tiered strategy was employed given the inconsistencies in the literature in terms of keyword identifiers. It was found that terms relating to occupational allergy and asthma combined with protein were the most productive for locating literature for non-oral routes of exposure; however, these terms were not universally effective and secondary searches were done on articles citing relevant publications and using additional keywords from these papers. Targeted secondary searches were also conducted on specific plants to locate exposure and potency information if a plant was mentioned as having an association with IgE-mediated responses by a non-oral route. The search was not limited by publication year and was completed at the end of 2014. A "cast a broad net" approach was used given the scarcity of literature, meaning that we included all literature that suggested a relationship between exposure to protein(s) of plant origin and evidence of IgE-mediated allergic responses and for which we could find some associated quantitative or semi-quantitative exposure information. For plant sources with sparse data we included all relevant citations, and for plant sources with large literature bases we focused on authoritative reviews, particularly those that included quantitative estimates of exposure and/or potency.

Baur and Bakehe (2014) used an evidence-based approach to evaluate the strength of the evidence for causality for occupational allergen exposures and asthma for plant, animal, and synthetic chemical allergens. Evidence was rated on a 7-point scale from no scientific evidence to strong evidence. Plant-derived allergens rated as having some evidence are listed below and were used for additional targeted literature searches for exposure/potency information:

Strong evidence: None

Moderate evidence: bakery (flour, amylase, and storage mites), detergent enzymes (noted for reference), latex, papain (exposure data lacking), paprika (most references refer to pollen from the plant, the spice allergy papers lack sufficient exposure data), psyllium, soybean (hulls and flour), tobacco (insufficient quantitative exposure information available), tea dust (green tea primary allergen not a protein), and Western red cedar (primary allergen not a protein).

Limited evidence: African maple, *Asparagus*, carnation, castor beans, bromelain of *Ananas comosus*, *Chrysanthemum*, Eastern white cedar, garlic dust, guar gum, gum Arabic, iroko tree, ispaghula husks, Madagascar jasmine, pine, porcini

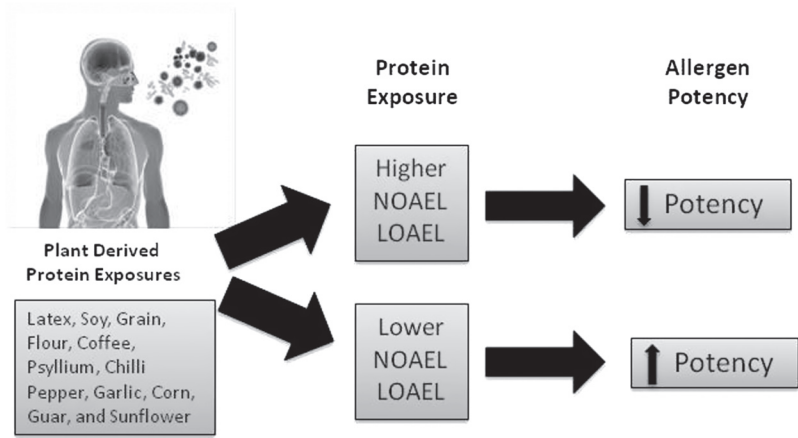


Figure 1. Process used for establishing the relationship of PEL settings and respiratory allergy potency.

mushroom, poppy, rose, rye flour, senna, sunflower pollen, various woods (fir, chestnut, framire, Mansonia, oak, obeche, walnut, white poplar, various flowers and weeping fig).

The criteria for further consideration in this evaluation were those allergens for which the primary allergenic components are known to be proteins and for which some quantitative measures of exposure that could be linked to allergen and/or protein content were available (Figure 1). The data used to make rough estimates of effect and/or no-effect levels for the plant materials are described in the following paragraphs and the qualitative estimates used to group the plant proteins into three potency bins are shown in Table 1.

Paprika/chili pepper

van der Walt et al. (2013) described occupational asthma in spice workers with a focus on pepper and garlic. Paprika allergens are well characterized, but quantitative occupational exposure data are lacking. Chili allergens are not well characterized (Schöll and Jensen-Jarolim 2004), but van der Walt et al. (2013) describe both dust exposure and the quantity of IgE-reactive allergen in the dust samples. Schöll and Jensen-Jarolim (2004) also point out that the role of capsaicin in increasing epithelial permeability in the gastrointestinal tract could play a role in allergenicity of chili. The impact of capsaicin on respiratory allergen dynamics has not been characterized. It is suggested that airway spasm due to chili

constituents plays a role in occupational symptoms in addition to IgE-mediated responses. The study population described in van der Walt et al. (2013) was spice mill workers exposed to a variety of spices; the only specific spices mentioned were chili, paprika, and garlic. Environmental measurements included inhalable dust and enzyme-linked immunosorbent assay (ELISA) determined airborne allergens for chili and garlic. Of note, fresh garlic and chili (species not specified) were used to prepare the extracts for ELISA development, it is known that there can be a change in allergen activity as a result of the drying process (e.g., for paprika) and it is presumed that the workers were exposed to dried spice material in the dust. In addition, it is unknown what the constituents of the dust were in addition to the chili and garlic allergens.

Protein was measured in the fresh extracts, but not in the dust samples. The mean dust levels were 2.06 mg/m³, with peak levels 48 mg/m³. Mean aerosolized chili pepper allergen was 0.44 mg/m³. Dust particulate correlated strongly with airborne chili pepper allergens. These authors concluded “spice mill workers exposed to inhalable spice dust particulate (mean > 2 mg/m³) containing garlic and chili pepper allergen levels of 0.24 mg/m³ and 0.44 mg/m³, respectively, have an increased risk of work-related lower respiratory symptoms, allergic sensitization, probable asthma, and obstructive lung disease.” It is likely that both immunological (IgE) responses to chili pepper in combination with non-IgE-mediated respiratory mechanisms are responsible. It is difficult to estimate the relevant protein content of the dust for comparison with other allergens where specific allergen was not measured. An estimate of the occupational LOAEL is 0.44 mg/m³ (as allergenic protein not total protein) as estimated by van der Walt et al. (2013). This value is so much higher than those for the other characterized protein allergens; we believe its placement in the low-potency category is justified despite all of the uncertainties in the calculations discussed above. An additional factor considered is the widespread use of chili pepper and the low number of reports.

Garlic

Several garlic allergens have been described that appear to play a role in occupational sensitization to garlic. There appears to be significant cross-reactivity between these antigens and

Table 1. Proposed potency classification of plant-derived protein materials based on occupational exposure assessment.

Plant-derived protein-containing materials	Estimated NOAEL protein exposure range	Projected potency classification
Chili pepper	> 100 µg/m ³	Low
Garlic		
Corn		
Guar		
Sunflower		
Buckwheat	> 0.1 – < 100 µg/m ³	Medium
Coffee		
Flour (Bakery)		
Grain (wheat, oat, and rye)		
Psyllium		
Soy		
Alcalase (reference)	< 0.1 µg/m ³	High
Latex		

constituents from related family members, most notably with onion, but also perhaps with *Asparagus* and shallot (van der Walt and Jeebhay 2010). The same study population described above for chili was co-exposed to garlic (van der Walt et al. 2013). Like chili, the ELISA was developed using fresh garlic proteins while the workers were exposed to dried garlic dust. Workers exposed to mean aerosolized garlic allergens at mean concentrations of 0.24 mg/m³ showed increased rates of respiratory symptoms and allergic sensitization. An estimate of the occupational LOAEL is 0.24 mg/m³ (as allergic protein, not total protein) as estimated by van der Walt et al. (2013). This value is so much higher than those for the other characterized protein allergens; we believe its placement in the low-potency category is justified despite all of the uncertainties in the calculations discussed above.

Psyllium

Psyllium is a known respiratory sensitizer and can trigger symptoms following ingestion (Freeman 1994). Case reports of occupational allergy from psyllium dust exposure have been reported for pharmaceutical, food processing, and health care workers (McConnochie et al. 1990; Marks et al. 1991; Malo et al. 1990a; Bardy et al. 1987; Bernedo et al. 2008) and confirmed to be associated with psyllium via skin prick testing (SPT) and determination of specific IgE. Arlian et al. (1992) examined the allergenic and antigenic components of psyllium seed. Psyllium husk contains small amounts of proteins (2.7%) that carry over during processing from the seed endosperm. Endosperm extract showed 7 different antigens with endosperm, embryo, and husk containing the same antigens but in different quantities. Psyllium husk was tested for its ability to induce respiratory sensitization in a guinea pig intra-tracheal test (unpublished data). This study showed that psyllium total protein was about an order of magnitude less potent than the benchmark laundry enzyme (Alcalase), resulting in setting an OEG at 150 ng/m³ psyllium protein (unpublished data) as calculated from the Alcalase OEG of 15 ng/m³ (Sarlo 1994). An estimate of the occupational NOAEL is 150 ng/m³ (as total protein).

Grain dust

Based on the information summarized below, the threshold limit value (TLV) is converted to total grain dust protein for the allergenic grain dusts (wheat, barley, and oat) of 2 µg/m³ and to 7.5 µg/m³ for the less allergenic grain dusts (soy, sunflower, and corn). These values are based on extractable protein, while the other reference values are based on total nitrogen. Total nitrogen may inflate protein values by including non-protein nitrogen, while extractable protein may underestimate total protein by excluding insoluble protein. Hence, the total protein exposure for grain dust may be higher than that reflected by extractable protein.

It has been known for many years that exposure to grain dust gives rise to respiratory problems (Williams et al. 1964; Skoulas et al. 1964; Chang-Yeung et al. 1985; Becklake 2007; Swan et al. 2007; HCN 2011; Omae 2007). Due to their potential to induce respiratory tract irritation and asthma, the US Occupational Safety and Health Administration (OSHA 1989) proposed to establish a 4 mg/m³ time-weighted average (TWA)

for dust generated from wheat oats and barley (grain dust). Similar occupational exposure limits (OELs) have been set in the Netherlands (1.5 mg/m³ as inhalable dust) (HCN 2011), Japan (1 mg/m³ as respirable dust and 4 mg/m³ as “total” dust; Omae 2007), and in the UK (10 mg/m³; Swan et al. 2007).

OSHA notes that grain dust is a complex mix of husk particles, cellulose, starch, spores, insect debris, pollen, rodent hair, and mineral particulates. Grain dust was historically regulated under the nuisance dust standards of 15 mg/m³. OSHA notes that respiratory symptoms are still experienced by workers at exposure levels below 10 mg/m³, even though symptoms are diminished compared with exposure at 15 mg/m³. The OSHA 4 mg/m³ standard was challenged by industry and trade associations as too conservative and difficult to implement, hence OSHA established a new 10 mg/m³ total dust limit as an 8-h TWA for wheat, oat, and barley dust. OSHA reports that grains other than those listed are regulated as generic particulates with a permissible exposure limit (PEL) of 15 mg/m³. However, the 4 mg/m³ standard that OSHA proposed based on a National Institute of Occupational Safety and Health (NIOSH) study of grain workers represents the best estimate of an exposure value that could be compared with the enzyme OEGs. OSHA indicates that the hazards of exposure to cereal dust have been known for a long time, being historically described as grain fever, a syndrome that includes wheezing, chest tightness, cough, eye and nasal irritation, and symptoms of chronic respiratory disease. Grain dust may also induce asthmatic reactions via an allergic mechanism particularly in atopic individuals. The NIOSH study that formed the basis of the OSHA 4 mg/m³ limit examined the respiratory health of approximately 300 grain handlers. Based on evaluation of dust exposures using personal samplers, this study reported that only 14% of the workers were exposed to dust levels (8-h TWA dust levels) greater than 5 mg/m³, while 7% were above 10 mg/m³. It was reported that workers showed a significant excess of cough and expectoration during a work shift (compared with time not at work) in which dust concentrations were below 5 mg/m³. At dust levels between 10 and 15 mg/m³, there was a significant increase in prevalence of wheezing and dyspnea during the work shift. In a three-year follow-up study, grain workers showed no greater decline in pulmonary function than could be accounted for by age alone. This study also reported that the types of grain dusts most likely to be associated with symptoms are wheat and barley followed by rye and oat. Less likely were corn, soybean, sunflower, etc. OSHA indicated that the dust limit would apply to flour exposures in bakeries and other workplaces.

The American Conference of Governmental Industrial Hygienists (ACGIH) (as cited by OSHA) had utilized data from the above study along with estimates of worker exposures that were subjectively estimated by the workers to support that 4 mg/m³ grain dust represented a NOEL. Despite criticisms, OSHA indicated that the results from the study are consistent with other reports that supported symptoms associated with grain dust exposures below 10 mg/m³; however, they note that there is a lack of definitive evidence that grain dust exposure results in chronic pulmonary disease despite the symptoms of chronic bronchitis and cough. A final standard was set at 10 mg/m³. OSHA notes that the least likely grain dusts to contribute to respiratory symptoms are corn, soybean,

sunflower (and others); these other grain dusts are regulated as particulates not otherwise regulated at a PEL of 15 mg/m³.

Mukherjee et al. (1998) measured grain dust exposures in two grain storage centers in Bangalore and also measured the protein content of the grain dust. They found that approximately 50% of the dust was in the respirable range. In the first center, the storage air dust measurements were approximately 1.5 mg/m³ while personal exposures were higher, approximately 10 mg/m³. In the second center, storage air showed approximately 7 mg/m³ dust while the personal exposures of loaders and sweepers were 15–20 mg/m³. The protein measurements were 0.15 µg/m³ for storage air at the first center (88 µg/g dust), whereas the protein measurements at the second center were 2 µg/m³ (740 µg/g dust). Based on these data from Mukherjee et al. (1998), grain dust is expected to have 0.01–0.1% protein. Hence, this would translate to a protein exposure of 0.4–4 µg/m³ at the proposed 4 mg/m³ TLV. In another study, Dashek et al. (1986) measured protein content in grain dusts from air sampling above conveyor belts in grain elevators and also in settled dust samples collected from beams and rafters in the grain elevators. Water-extractable protein was barley (0.1%), corn (0%), flax (0%), oat (0.08%), wheat (0.1%), and settled dust (0.065%), consistent with the values reported by Mukherjee et al. (1998). Of note, these values are extractable protein and therefore may underestimate total protein by an unknown factor. DoPico et al. (1977) demonstrated the acute effects of grain dust exposure during an 8-h work shift on 248 workers. They found a dose-related acute adverse effect on lung function irrespective of smoking habit, atopic status, or age. The mean total dust level was relatively low, 3–7 mg/m³, which is consistent with OSHA and NIOSH recommended safe levels. More recent reports are also supportive of these values (Farant and Moore 1978; Senthilselvan et al. 2010; Dimich-Ward et al. 2011). An estimate of the NOAEL is 4 mg/m³ total dust for wheat, barley, rye, and oats or 2 µg/m³ grain protein. An estimate of the NOAEL for the less allergenic grain dusts cannot be made based on the PEL of 15 mg/m³ total dust for other grains such as corn and sunflower. This PEL is not based on protection from allergenic responses, it is a default for inert or nuisance dusts. Corn is the most widely grown cereal grain (839 million tons in 2012) (Pechanova et al. 2013). Despite widespread use, there are few reports of respiratory allergy to corn proteins (this contrasts with soy for which there are many reports of respiratory allergy that suggest a higher allergenic potential). Maniu et al. (2010) described one case of IgE-mediated allergy attributed to inhalation exposure to corn starch and cited the limited available allergy literature. A more in-depth search for information on IgE-mediated hypersensitivity to corn protein revealed additional information on the putative oral allergens, but no additional data relevant to non-oral exposure. We place corn in a low-potency allergenicity group based on its widespread use and the paucity of reports attributing type-1 allergic responses to inhaled corn proteins. Sunflower seed represents a similar situation in that there are few reports of IgE-mediated allergic responses following inhalation exposure. Sunflower pollen is clearly allergenic and sunflower seeds can result in IgE-mediated allergic responses following ingestion or inhalation. Sunflower seeds are widely used as livestock and bird feed and are processed for oil used for edible oil and margarine production (Besler

et al. 2001; Vandenplas et al. 1998). We group sunflower in a low-potency allergenicity group based on the limited reports attributing type-1 allergic responses to sunflower seed proteins, however, with less confidence than that for corn due to lower volume of production (40 million tons of seeds globally, National Sunflower Association).

Buckwheat

Buckwheat is an occupational allergen (Wieslander 1996; Gohte et al. 1983). European expert groups have not included buckwheat in discussions of cereal grain occupational standards because the buckwheat plant is from a different taxonomic group than the cereal grains. Gohte et al. (1983) show that exposures comparatively to low levels (1–2 µg/m³) of buckwheat dust are associated with a risk of occupational allergy and the protein constituents are implicated (Wieslander 1996). The Gohte et al. (1983) study is small and the only study that reports buckwheat dust levels and associated SPT, radioallergosorbent test (RAST), and symptomology so this is not a robust quantitative estimate. However, there are numerous reports of allergic responses following both inhalation and ingestion of buckwheat. Five protein allergens have been identified, including albumin, vicilin, two trypsin inhibitors, and a globulin (Heffler et al. 2014) but the concordance between major allergens for oral versus inhalation exposure is not known. The estimated protein content of buckwheat is 10% (Hong et al. 1996). An estimate of the LOAEL is 2 µg/m³ total dust or 0.2 µg/m³ protein.

Flour dust

The linear relationship between exposure to flour dust, allergenicity, and sensitization symptoms has been investigated in several studies (ACGIH 2001; Heederick and Houba 2001; Del Moral et al. 2007; Tatham and Shewry 2008; DECOS 2004; SCOEL 2008; Musk et al. 1989; Cullinan et al. 1994; Nieuwenhuijsen et al. 1994, 1999; Nielsen et al. 2012; Tee et al. 1992; Baatjies et al. 2010; Bulat et al. 2004; Page et al. 2010). Flour dust comprises the finely milled and processed grains of mainly wheat, rye, millet, barley, oats, corn cereals, or a combination of these grains. The levels of flour dusts in traditional and industrial bakeries and job types have been studied (Baatjies et al. 2010; Bulat et al. 2004). Tatham and Shewry (2008) report that mature grains of these cereals can contain over 1000 different proteins and at least 40 different allergens with a potential for cross-reactivity. ACGIH (2001) proposed a TLV for flour dust of 0.5 mg/m³. In this document they note that flour dust contains generally 10% protein, hence total mixed protein exposures would be 0.05 mg/m³. This value is assumed to be based on total nitrogen since it is consistent with the food protein values of flour, and food protein values are generally determined using total nitrogen techniques. The ACGIH study further noted that sensitization occurred at dust levels as low as 0.5 mg/m³, hence it was considered the critical effect and the value used to establish the OEL to minimize sensitization. Heederick and Houba (2001) provided a more extensive analysis of wheat dust dose–response curves and reported that wheat dust contains an average of 1.45 µg wheat allergenic protein/mg flour dust. They also concluded that the NOEL for induction of sensitization to wheat flour dust was

between 0.5 and 1 mg/m³ inhalable dust. Wheat flour contains α -amylase, one of the major allergens, at a concentration of 0.1–1 mg/g flour. The TLV of 0.5 mg/m³ would equate to 50 μ g/m³ protein or 725 ng/m³ total wheat allergens and exposure to 50–500 ng/m³ α -amylase.

The Dutch Expert Committee on Occupational Standards (DECOS 2004) also conducted a comprehensive data collection and evaluation of the allergenicity of various flour dust cereals. They also reported sensitization as the critical endpoint, which often preceded the onset of allergic symptoms, thus preventing sensitization prevents the onset of symptoms. Significantly, they observed that sensitization was exposure dependent, but no threshold could be identified. Hence, they used a linear non-threshold extrapolation to determine the probabilities for sensitization. They estimated the 0.1%, 1%, and 10% additional risk of sensitization corresponded to 0.012, 0.12, and 1.2 mg/m³, respectively, of inhalable flour dust for an 8 h per day/5 days per week lifetime exposure.

Other studies have also reported increased risks of nasal- and asthmatic-like symptoms at flour dust concentrations ≥ 1 mg/m³ (SCOEL 2008; Page et al. 2010), which is consistent with the findings reported in ACGIH 2001 and DECOS 2004. Flour dust exposures as low as 1–2.5 mg/m³ are associated with a significantly elevated risk of sensitization to wheat allergens. Using logistic or linear regression, Musk et al. (1989) studied 279 workers of a modern bakery and found that immediate-type skin prick test responses to wheat and symptoms, lung function decrease, and bronchial reactivity related to current or past exposure. Additionally, Page et al. (2010) have determined prevalences of sensitization to bakery-associated antigens or BAAs and work-related respiratory symptoms at a large commercial bakery. The estimated geometric mean for inhalable flour dust concentrations for the lower-exposure group was 0.235 mg/m³, and for the higher-exposure group was 3.01 mg/m³. The sensitization prevalence to α -amylase was 0% and 6%, respectively, and sensitization to wheat 6% and 27%, respectively, which is also consistent with the ACGIH (2001) and the Scientific Committee on Occupational Exposure Limits (SCOEL) (2008) studies. Cullinan et al. (1994) also reported work-related symptoms in employees exposed to flour in bakeries that were related to exposure intensity. They found that the prevalence of sensitization to wheat flour allergens was higher than the prevalence of sensitization to the added enzymes, suggesting that sensitization is largely dependent on the flour dust levels and adverse effects occur at levels about 2 mg/m³. An estimate of the NOAEL is 0.5 mg/m³ total dust or 50 μ g/m³ protein or 725 ng/m³ total wheat allergens.

Cotton dust

NIOSH has recommended an OEL for cotton dust targeted to protect against byssinosis (an airway obstructive disease), not allergenicity. Quirce and Bernstein (2011) categorize byssinosis as an occupational asthma that is not allergen mediated. It was later shown that byssinosis was primarily due to exposure to endotoxins from the fibers of raw cotton (Eduard et al. 2012). While the protein and enzyme content of cotton provide a plausible basis for the induction of immediate hypersensitivity reactions, data that clearly show a role for type-I

hypersensitivity responses and health effects in cotton workers are not available and no data are available that could be used to separate endotoxin responses from cotton protein responses (De Olano 2009 Atkins et al. 1988).

Coffee dust

Coffee dust exposure is clearly associated with occupational asthma. There has been controversy concerning the relative contributions of endotoxin, chlorogenic acid, and contaminating castor bean allergens; however, the weight of evidence supports that coffee bean proteins play a role as occupational allergens (Layton et al. 1965 and 1968). Coffee contains arabinogalactan protein complexes which constitute about 15% of the dry weight of the beans. Proteoglycans have been characterized in a wide range of higher plants (Redgwell et al. 2005). The protein content of the various arabinogalactans from coffee varies from 0.5 to 2% (Redgwell et al. 2002). Interestingly, *Acacia* contains similar proteoglycans and *Acacia* gum has been associated with occupational asthma (Sander et al. 2006). Coffee beans contain other proteins, including enzymes, which have been proposed to be important in the redox state of the bean (Montavon and Bortlik 2004). However, there are no quantitative data on the concentration of enzymes or individual proteins in coffee beans. The first coffee bean protein allergen has been isolated and sequenced (Manavski et al. 2012). This protein is class-III chitinase of *Coffea arabica* from green coffee beans. Importantly, these authors also note the issues with assessing coffee bean allergy using coffee bean extracts, specifically, a lack of standardization in terms of antigens and potency of the commercial extracts and the presence of vasodilatory substances in fresh extracts which can result in false-positive skin reactions. While there are many studies documenting symptoms in coffee workers, there are few studies that both report measured dust levels along with data demonstrating sensitization to coffee.

In the early 1980s a study of plant workers involved in the processing of green coffee dust was published (Jones et al. 1982). This study examined 372 workers in two coffee plants in New Orleans. They reported a significant interaction between exposures to green coffee dust and years of employment that was related to reduced baseline expiratory flow rates (often used as a measure of asthma) compared with workers exposed to roasted coffee. However, only small numbers of employees worked in green coffee exposure areas. Positive RAST tests to green coffee bean extracts were also associated with reduced lung function, although there were few symptoms and lung function effects were modest. The authors concluded that their data demonstrated the allergenicity of green coffee dust and demonstrated an adverse effect of occupational exposure to unroasted coffee. Mean total dust levels in the green coffee areas were reported to be approximately 0.5 mg/m³ (no indication of variability reported). Larese et al. (1998) reported on the sensitization to green coffee bean and castor bean allergens in 112 workers in a modern coffee plant in Italy. Sensitization to green coffee bean was found in 26% of the green coffee workers, 2.7% of the roasted coffee workers, and 4.5% of the office workers. Castor bean sensitization was seen in 3 of 10 workers sensitized to green coffee bean. There was a significant correlation between sensitization to green coffee bean and work-related symptoms. Exposure was characterized as

total dust concentrations with measured values of 0.07–0.3 mg/m³ in the green coffee processing area and 0.08–1.75 mg/m³ in the roasted coffee area. These values were based on seven 2-h air samples in each area. A geometric mean of 0.15 mg/m³ is reported, but this is apparently across both the green coffee and roasted coffee areas.

There are no measured values for protein content of green coffee bean dust; however, the protein content of the beans has been reported by Franca et al. (2005). These authors report on the protein content of several varieties of coffee. Protein content was estimated from total nitrogen levels as approximately 15%. It is difficult to choose an exposure level from these data to use for the potency ranking since studies with better exposure metrics failed to clearly demonstrate sensitization to coffee (versus castor bean, endotoxin, etc.). The value of 0.5 mg/m³ has been chosen, which was the measured mean value from the Jones et al. study, given the evidence of sensitization to green coffee in both this study and in the Laresse et al. (1998) study which measured exposure concentrations of less than 0.3 mg/m³. This would translate to a protein exposure of 75 µg/m³ coffee protein and would put exposure to coffee dust protein in the same general range as exposure to flour dust protein at the TLV. Although the flour dust TLV has been estimated to be a NOEL for sensitization, there are insufficient data to conclude that the effects of coffee dust exposure at this concentration are more severe than the effects of flour dust exposure given the paucity of air measurements that the comparisons are based on and the small populations monitored. The data are sufficient to support that coffee dust is not likely to be less potent than flour or grain dust by a large margin when adjusted for protein content and also to conclude that coffee dust is not a highly potent allergen but falls within the general potency range of the common plant-based occupational bioaerosols. An estimate of the occupational NOAEL is 0.5 mg/m³ dust and 75 µg/m³ total protein.

Latex

Baur et al. (1998) proposed that a level of 0.6 ng/m³ total latex allergen protein represents a NOEL for elicitation of symptoms in sensitized workers. Baur et al. (1998) do not report airborne total latex protein concentrations. Total dust levels were measured in various areas of the hospital, but there was no correlation with latex allergens. Howell et al. (2002) reported that extracts from latex gloves contained from 0 to 946 µg/g total protein and from 0.002 to 112 µg/g allergenic proteins supporting that about 10% of the total protein is allergenic. There are a number of latex allergens; however, there appear to be two predominating allergenic proteins. The two major allergens for health care workers are the 4.7-kDa Hev b 6.02 and 14.6-kDa Hev b 1 (Posch et al. 1998). An estimate of occupational NOAEL is 6 ng/m³ protein or 0.6 ng/m³ latex allergenic protein.

Soybean proteins

Environmental exposures to soy associated with symptoms appear to be lower than occupational exposure levels for other plant proteins, but the circumstances of diagnosis and measurement are so divergent from the other occupational exposure datasets that direct quantitative comparisons are difficult.

Soy is a significant food allergen. Soy flour has been associated with occupational and environmental allergy following inhalation and is also a significant food allergen. In Spain, soy dust has been associated with environmental allergy. The association of soy dust with asthma in Barcelona is particularly interesting because a significant amount of work was conducted to document air levels of soy allergen associated with symptoms and to reduce soy dust exposures to levels not associated with significant health effects.

The allergens involved in occupational asthma caused by soybean flour are predominantly high-MW proteins that are present both in soybean hull and flour, and they are different from the allergens causing asthma outbreaks, which are mainly relatively low-MW proteins concentrated in the hull (Quirce et al. 2000). Soybean hulls contain three main allergens with MWs of 8, 7.5, and 7 kDa (Codina et al. 1999). The major allergen causing the epidemics in Barcelona, Spain, was a glycopeptide less than 14 kDa molecular mass abundant in soybean dust. This allergen occurs in all parts of the soybean plant at all stages of growth, but the telae (hulls) and pods are by far the richest source (Swanson et al. 1991). Airborne soybean dust has the potential to cause asthma in allergic individuals. The frequency of soybean dust allergenicity in the Barcelona population has been estimated at approximately 1 per 1700 persons. The principal allergen appears to be an 8-kDa protein that occurs on the seed surface (Gly m 1) or HPS (hydrophobic protein from soybean). González et al. (2000) using an ELISA method show that similar responses in the ELISA Gly m1 assay were found when using 0.125 µg/ml of soybean hull extract compared with 0.02 µg/ml of Gly m1. This suggests that about 15% of the soy hull protein is Gly m1. They further report that Gly m1 accounts for about 75% of the total IgE-binding activity of whole soybean hull extract. Using these values it can be estimated that during asthma outbreaks in Barcelona the mean allergen values on epidemic days of 1.6–10 ng/m³ allergenic soy proteins corresponded to 1.2–7.5 ng/m³ Gly m1 and to 8–50 ng/m³ soy protein. The values on non-epidemic days would have corresponded to 0.000225–0.225 ng/m³ Gly m1 or 0.0015–1.5 ng/m³ of soybean hull protein. The corresponding values for the days that sensitized subjects reported elicitation of symptoms would correspond to 0.2–0.4 ng/m³ allergenic soy protein or 0.15–0.3 ng/m³ Gly 1 or 1–2 ng/m³ soy hull protein. Note that OSHA considers soybean grain dust to be less likely to induce adverse respiratory effects in occupationally exposed workers than oat, barley, and wheat and does not include soybean dust in the grain dust TLV; soybean dust is regulated as a nuisance dust.

Soybean plant workers are sensitized to different soybean allergens than those associated with community responses (Cummings et al. 2010). Soybean plant workers associated with production in the further processing of soy flake and production support were exposed to higher amounts of dust and soybean allergens and showed an association with asthma and soybean sensitization while non-production/production support workers did not. The job classifications associated with allergic symptoms were exposure to dust and soybean allergen at levels ranked as medium or high, while other workers had exposures in the low categories. The distributional tertiles of inhalable dust concentration were reported as high, 0.75–1.6 mg/m³; medium, 0.58–0.73 mg/m³; and

low, 0.17–0.54 mg/m³); distributional tertiles of inhalable soy antigen concentration were reported as: high, 2,635–25,958 ng/m³; medium, 959–2,297 ng/m³; and low, 24–804 ng/m³). As a rough estimate the upper values from the lower tertile has been selected to represent the NOAELs. An estimate of the occupational NOAEL is 0.5 mg/m³ (total dust) or 800 ng/m³ allergenic protein.

Guar

There are published data supporting that Guar proteins are occupational allergens (Baur and Bakehe 2014), but there are no published exposure/effect data. An internal OEG for guar was established to be 30-fold higher than the Alcalase OEG-based animal test data for mixed guar proteins (P&G unpublished data). However, this OEG may be very conservative given that clear responses were not seen in the historical animal test data suggesting that the effective concentration may have been significantly higher than the highest dose tested. Indirect support for a low potency for Guar proteins in IgE-mediated allergy is the widespread use of guar in the pharmaceutical, food, and cosmetic products industry with few reports of an association with allergy (Gultekin and Doguc 2013; Tripathy et al. 2013). In addition, Malo et al. (1990b) describe the working conditions of the dye worker population that provides the basis for the best-described cases of occupational IgE-mediated allergy to guar. Although quantitative measures of airborne guar are not provided, the work process involves pouring guar from buckets from a higher level in the factory into large vats resulting in aerosolized guar. In addition, the authors hypothesize that additional guar may be aerosolized by working with the fibers after processing in the vats. These working conditions suggest that workers may be exposed to high amounts of guar dust. Collectively, these observations support categorization of guar protein on the lower end of the potency scale.

The way forward

There are no validated or widely accepted test methods for determining the allergenic potency of novel or unstudied proteins for respiratory allergy potential. There is, however, considerable understanding of the cellular and molecular mechanisms through which IgE antibody-mediated allergy develops, and this understanding can support and inform the development of new and improved approaches to safety assessment. There is an appreciation that the pivotal event in the acquisition of sensitization is the induction of an IgE antibody response. Such antibodies distribute systemically and arm mast cells and basophils. Following subsequent exposure to the same allergen, IgE antibodies bound to mast cells/basophils are cross-linked and this causes cellular activation, degranulation, and the release of mediators (including vasoactive amines) that together drive the inflammatory response and an allergic reaction.

An important aspect in identifying and characterizing hazards associated with proteins is that immune responses to foreign (non-self) proteins can be immunogenic only, or they can be immunogenic and provoke the class of immune response required for allergic sensitization; in this case a T helper 2-type immune response that supports the elaboration of IgE antibody. Clearly, it is of considerable importance to

distinguish carefully between immunogenicity and allergenicity. This is facilitated by our increasing understanding of the ways in which the adaptive immune response is tailored to meet different forms of challenge. At the heart of this is the functional heterogeneity among T lymphocytes, and it is the balance between functional subpopulations of T helper cells that determines the qualitative outcome of an immune response. In the context of this article it is the pattern of T-lymphocyte responses that will determine whether exposure to a foreign protein will result in immunogenicity or allergenicity.

Certainly against this background, it is very unlikely that in the short and medium term there will be any prospect of designing a stand-alone method that does not rely on the use of animals. When considering a path forward without animal testing, it is unlikely that a single test method will be able to determine whether a protein of interest has allergenic potential of concern in the context of specific consumer product exposure risk assessment. It is therefore more realistic to consider that future safety assessments will depend upon the availability of a suite of approaches that bring together various sources of information that can be used collectively to provide a weight of evidence evaluation of allergenic potential. This has been recognized in the context of non-animal test methods for other endpoints. The vision for animal alternative approaches is transitioning from a single test to define hazard to the development of multi-approach tool boxes. This vision is outlined in the context of “Integrated Approaches to Testing and Assessment” (IATA) and is defined as structured approaches that integrate and weigh different types of data for the purposes of performing hazard identification and hazard characterization (Tollefsen et al. 2014). The information that is likely to populate such a “tool box” for protein allergens will doubtless include consideration of the physicochemical properties of the protein sequence and/or structural homology with known protein allergens, and possibly the display of discrete T-cell epitopes. In addition, the nature of interactions between proteins and DC, and the way in which proteins are recognized, internalized, processed, and presented by the immune system may also be informative. Thus, a suite of approaches will be needed; to be useful for cosmetic ingredients, these approaches will need to be based on *in silico/in chemico/in vitro* methods consistent with various recent legislation that either bans use of data from *in vivo* animal studies or encourages a move to methods for safety assessment that obviate the need for experimental animals.

To develop robust and relevant methods for assessment of the respiratory sensitizing potential of proteins, it will be necessary to have access to reference respiratory allergenic proteins that can serve as positive controls representing a range of sensitizing potencies. This approach has been used successfully for the development of alternative methods for skin sensitization. Benchmark chemicals of different potencies were assessed in the local lymph node assay (LLNA), a murine test method that has largely replaced the previous guinea pig methods for the prospective identification of skin-sensitizing chemicals (Gerberick et al. 2005; Kern et al. 2010). Accordingly, the LLNA database is now being used as a key resource for the development of *in vitro* methods that may in the future be useful for characterization of the relative skin-sensitizing potency of contact allergens (e.g., Natsch

et al. 2015). Of course, it is important to acknowledge that the ultimate benchmark for authentication of potency predictions must be the actual relative potency in humans. In this article, we have attempted to categorize the potency of protein respiratory allergens by reference to occupational exposures to plant proteins. This approach is not without limitations in that it is only semi-quantitative at best, but it does provide investigators with a starting point for evaluating novel approaches with respect to their relevance, sensitivity, and specificity. The ultimate goal is to use such information on the relationship between exposure and potency to assist in the development of a strategy for more accurate risk assessments. We see this list of plant proteins as a starting point in the development of plant protein allergen reference materials. We anticipate that in the process of using these plant materials and/or their associated proteins in the development of test methods the preliminary potency estimates suggested here will be refined and the list of candidate reference plant proteins will be expanded.

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Declaration of interest

This review was performed during the normal course of the authors' affiliation or employment as shown in the first page. None of the authors have participated in legal or regulatory proceedings during the past 5 years on the subject matter of the paper. The authors have sole responsibility for the writing and content of the manuscript.

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