Are we closer to developing threshold limit values for allergens in the workplace?

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The use of immunoassays has facilitated the measurement of high molecular weight sensitizers, usually protein molecules, in the picogram and nanogram per cubic meter range. This facilitated the evaluation of exposure response relationships for bakery workers, exposed to wheat allergens and fungal α-amylase and other groups exposed to other allergens such as laboratory animal workers. The application for the standard setting is still limited and requires rigorous standardization, but can be expected in the near future. Curr Opin Allergy Clin Immunol 1:185–189.

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Abbreviations

ELISA	enzyme-linked immunosorbent assays
HMW	high molecular weight
TLV	threshold limit value

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Introduction

Asthma is one of the most common causes of chronic ill health. It is generally accepted that asthma is a disease in which exposure to chemical and biological agents, such as allergens and irritants, plays an important role [1]. Sensitization against environmental allergens is an important underlying mechanism in the development of asthma. Prevalence studies among occupational groups such as grain workers, bakery workers, and laboratory animal workers exposed to sensitizing agents show prevalence rates of an order of magnitude of 5–50% [2–4,5••,6–8,9••,10••].

A distinction is usually made between chemical sensitizers, usually of molecular weight such as toluene diisocyanate and glutaraldehyde, and so-called high molecular weight (HMW) sensitizers, proteins or glycoproteins that can provoke a specific IgE response in workers exposed to these agents. Molecular weights of HMW sensitizers are typically in the 5000–70 000 M_r range. This review will be limited to HMW sensitizers, because the mechanism by which exposure to low molecular weight sensitizers leads to either sensitization or asthma is often unclear, and can differ from IgE-mediated mechanisms described for HMW sensitizers.

Several agricultural products and animal excreta contain HMW sensitizers. Well known HMW sensitizers are wheat (*Triticum* sp.) proteins, rat and mouse urine proteins, latex (*Hevea brasiliensis*) and enzymes such as the baking additive fungal α-amylase usually derived from *Aspergillus oryzae*. Most of these agents contain several allergens. For instance, in wheat, more than 40 water soluble allergens have been described [11–13], whereas commercially available fungal α-amylase extracts contain one major allergen, *Asp o II*, and one or two other components to which workers can develop IgE antibodies [14,15].

Exposure control has usually been based on trial and error approaches because safe levels below which the allergic disease does not develop have not been identified for most allergens. As a result, few health-based exposure standards for exposure to aeroallergens in the air have been developed. For subtilisin, a bacterial enzyme used widely in detergents and a well-recognized respiratory sensitizer, originally produced from *Bacillus subtilus*, a threshold limit value (TLV) of 0.06 $\mu g/m^3$ for workplace airborne exposure has been proposed by the American Conference of Governmental Industrial Hy-

gienists [16]. However, there is considerable doubt about the underpinning of this TLV, and the proposed value seems to be determined mainly by analytical limitations, i.e. by the detection limits of some of the earlier methods for exposure measurements. An evaluation by the Nordic Expert Group for Criteria Documentation indicated that the TLV for subtilisin probably does not protect against sensitization [17]. A recent study [18] showed that sensitization occurs at exposure levels well below the TLV, although sampling and analysis of the enzyme levels have not been described in great detail in the paper.

Two reasons for this absence of health-based occupational exposure limits can be given:

1. There has been a widespread belief that exposure response modelling for sensitizers is technically impossible, even when personal exposure data are available [19,20••]. The argumentation is based on the heterogeneity in response between individuals associated with differences in underlying mechanisms. However, several epidemiological studies have been able to demonstrate the presence of exposure–sensitization and exposure–symptom relationships.

2. Few measurement techniques existed until recently that made it possible to measure the allergens directly. In some of the early measurement series, taken in the framework of epidemiological studies, exposure to wheat has been assessed by classic total dust measurements. Latex allergen exposure has in some instances been evaluated by measuring the protein content of the dust [21,22]. Enzyme exposure could only be evaluated by using functional assays that measured enzyme activity through substrate conversion, but did not measure the allergen itself. These approaches were often not sensitive or specific enough because other dust or protein sources were present in the same work environment, or for enzymes other enzymes in the dust, not responsible for sensitization, were able to convert the same substrate. As a result, few studies focused on establishing exposure–response relationships.

It is now well recognized that immunoassays using specific antibodies against the (epitopes of) HMW sensitizers may, in most cases, be the most suitable, sensitive and specific techniques for measuring allergen exposure levels [23••]. In particular, enzyme-labelled reagents (enzyme-linked immunosorbent assays; ELISA), in combination with chromogenic substrates are commonly used. In so-called sandwich assays the allergen to be measured is captured between the antibody-coated surface and the detecting antibody, which is then also directed to the allergen itself. In inhibition assays, the concentration of allergen in a dust extract is quantitatively determined as the capability to inhibit the binding of anti-allergen antibodies to an allergen-coated surface. An important feature of both approaches is that the activity of a small amount of allergen can be quantitatively detected without interference from many other agents usually present in dust samples. Since the introduction of immunochemical techniques, a considerable number of exposure studies have been performed in a wide range of occupational environments. Examples exist for the evaluation of allergen exposure to enzymes such as papain in the meat processing industry [24], fungal α-amylase in the baking industry [25], exposure to egg protein in the food processing industry [26], pig and cow urinary and dander proteins in agriculture [27,28], wheat allergens in bakeries and flour mills [13,29], rat and mouse urinary allergens in laboratories [30], and latex in the healthcare industry [21,22]. The technique has also been used to evaluate the allergenicity of different particle size fractions. Studies in bakeries [25] showed that larger particles in particular contain allergenic wheat proteins and fungal α-amylase. Studies in the baking industry [10••,25,29,31–34] showed that immunoassays are useful in characterizing the exposure to wheat flour and α-amylase allergens in personal dust samples. For several occupational titles clear differences in airborne allergen exposure existed in the above-mentioned studies, in which no differences in dust exposure levels could be found. The studies [10••,25,29] showed that the correlation between dust and wheat allergen and fungal α-amylase levels is poor to moderate.

Application of immunoassays in various occupational environments has led to the unravelling of exposure–response relationships for specific IgE-mediated sensitization and exposure-related allergic symptoms for a variety of allergens. Several publications exist with detailed information about exposure levels to allergens and quantitative exposure response relationships. Most exposure–response relationships have been described with sensitization as an endpoint. Quantitative exposure–response relationships are available for fungal α-amylase [7,10••], rat urinary proteins [5••,6] and wheat allergens [8]. All these studies showed a clear increase in sensitization risk with increasing aeroallergen exposure levels. For example, the risk of developing sensitization to rats (laboratory animal allergy) has been found to be positively associated with the level of allergen exposure. Data from three independent studies among laboratory workers were pooled into a large cross-sectional study as part of a European collaborative project [5••]. Data came from three cross-sectional studies in the Netherlands, the UK and Sweden. Selection criteria were harmonized, and this resulted in a study population of 650 laboratory animal workers (60.5% women) with less than 4 years of

exposure. Air allergen levels were assessed previously in each country and converted arbitrarily to Dutch allergen levels on the basis of an inter-laboratory allergen analysis comparison [35••,36]. Available sera were analysed for the presence of specific antibodies against common allergens (house dust mite, cat, dog, grass and birch pollen) and work-related allergens (rat and mouse urinary proteins). The analyses showed that average exposure multiplied by the number of hours worked per week with rats was more strongly associated with sensitization than average exposure or the number of hours worked with rats alone. Sensitization rates increased with increasing air allergen exposure. An elevated risk was observed at very low exposure levels. Atopic workers exposed for only a few hours per week with low exposure levels between 0 and 0.5 ngEQ/m^3.hr/week (exposure category arithmetic mean exposure 0.18 ngEQ/m^3.hr/week) had a more than threefold likelihood of being sensitized than non-exposed workers. Atopic workers in the highest exposure category with exposure levels above 8 ngEQ/m^3.hr/week had an almost fourfold increased sensitization risk, but their average exposure was more than 1000-fold higher than observed for the lowest exposed category (exposure category arithmetic mean exposure 188 ngEQ/m^3.hr/week). For atopic individuals the risk thus increased little with increasing exposure, whereas for non-atopic individuals a steadily increasing risk was observed. These results suggest that the lowest exposure levels observed seem sufficient to sensitize a considerable proportion of the atopic individuals, whereas the risk for non-atopic individuals to become sensitized at these levels was almost negligible and became noticeable only at higher exposure levels. Similar observations are available for wheat and fungal α-amylase, also with usually steeper exposure sensitization relationships for atopic compared with non-atopic individuals. A detailed analysis using advanced statistical smoothing techniques was applied to evaluate the shape of the exposure response relationship in greater detail [37]. The study showed a strong increase in the exposure–sensitization relationship at low exposure levels. The sensitization risk levelled off and even declined at higher levels, suggesting the influence of health selection as a result of the healthy worker effect. This pattern became stronger when sensitization in combination with the presence of symptoms was used as the endpoint in the statistical analysis.

Some studies evaluated other endpoints. For instance, Brisman *et al.* [9••] evaluated an exposure–response relationship for self-reported asthma and rhinitis, in a retrospective cohort study among 2923 Swedish bakery workers. The risk of asthma seemed to be increased at inhalable dust concentrations above 3 mg/m^3 (dough making or bread forming), whereas the risk of rhinitis was increased at all concentrations above 1 mg/m^3. A disadvantage of using questionnaires only to define the presence of asthma or rhinitis is that no distinction can be made between asthmatic and rhinitis symptoms with an immunological background or those caused by other mechanisms. Especially in the case of respiratory symptoms in bakers, a considerable proportion of symptomatic bakers show no sensitization to baking allergens, and other causal mechanisms for the development of their symptoms have been suggested [3]. Nevertheless, these results demonstrate the potential of well-designed epidemiological studies with strong exposure assessment strategies. Houba *et al.* [8] attempted to evaluate exposure symptom relationships in bakery workers by making a separate analysis for wheat-sensitized and non-wheat-sensitized workers. Their results were indicative of a somewhat steeper relationship in sensitized bakers compared with non-wheat-sensitized bakers. The study population was too small to make a further distinction between atopic and non-atopic bakers within the group of non-wheat-sensitized bakers. However, their study is indicative of the existence of exposure–symptom relationships next to exposure–sensitization relationships. This corroborates the view that exposure to allergens will first lead to sensitization and subsequently to symptoms. However, few attempts have been undertaken in epidemiological studies to try to study these relationships separately.

Recent studies also show that several allergens, such as rat urinary proteins and fungal α-amylase, appear to be very potent allergens, and are already associated with increased sensitization rates at exposure levels in the nanogram per cubic meter range for as little as a few hours per week [5••,7,10••]. Other allergens such as wheat proteins seem less potent, and sensitization rates increase when exposure occurs in the low microgram per cubic meter range [8]. Clear-cut exposure–response relationships in humans have as yet not been observed for latex proteins and many of the allergens present in agricultural environments. For latex this can be explained because few epidemiological studies on latex sensitization have so far been conducted in which including exposure was assessed with the use of sensitive latex-specific immunoassays. Similar exposure–response relationships have been observed for common allergens from the house dust mite and cats, but usually the exposure is measured on the floor, the major reservoir, instead of in the air because of detection issues and the fact that most particles remain airborne for a very brief period because of their large particle size [38–40].

Use of recent data for standard setting

Despite these promising applications of epidemiological study results, risk assessment for sensitizers has some major conceptual and practical pitfalls that need to be

solved in the near future [41,42]. First, there has been discussion about the relevant health endpoint that should be used in a risk assessment. Baur *et al.* [41] mentioned in their paper on TLV for sensitizers that asthma should be the endpoint of relevance for risk assessment. This restriction excludes other endpoints, which could also be considered adverse, such as occupational allergic rhinitis, and that appear in a considerably higher proportion of exposed workers [43]. The major reservation against using sensitization as an endpoint for risk assessment is that it is not considered as to be 'disease' [44]. On the other hand, there is widespread agreement that sensitization defined as the presence of specific IgE antibodies is the first step in a disease process that is accompanied by symptoms, bronchial hyper-responsiveness, and airway obstruction when exposure continues [45,46]. In addition, most studies have demonstrated a strong correlation between work-related sensitization and symptoms, suggesting that most sensitized workers are symptomatic. However, the correlation between sensitization and symptoms is not perfect, and most authors believe that symptoms can also be caused by non-immune-mediated mechanisms. Longitudinal data from an Italian study [47••] demonstrated that asymptomatic non-sensitized workers have an increased risk of developing symptoms during follow-up, underpinning the important predictive value of specific work-related sensitization.

Despite these scientific discussions regarding some procedural and technical aspects, exposure standards have been proposed for wheat dust using recently obtained information about exposure sensitization relationships for wheat dust. The study by Houba *et al.* [8] has been referred to by the American Conference of Governmental Industrial Hygienists to underpin their proposed TLV of 0.5 mg/m^3 time weighted average exposure over a work-shift (8 h) [48••]. Another study [49••], using the same data, is in preparation by the Dutch Expert Committee for Occupational Exposure Standards. Interestingly, in both risk assessments, the exposure threshold has been expressed as exposure to wheat dust in the air. This was necessary because immunoassays for the measurement of wheat allergen levels are not available for hygienists in the field, and wheat flour allergen exposure has to be evaluated by measuring inhalable dust levels as a proxy. However, for allergens that are more potent and sensitize at nanogram per cubic meter levels, such as rat urinary proteins and fungal α-amylase, expressing exposure in terms of dust levels is not appropriate, because the relationship between allergen and dust levels is usually extremely poor, and significant sensitizing allergen levels may often have been encountered when dust levels are below the detection limit of conventional respirable and inhalable dust sampling. Therefore, for most HMW allergens, the exposure assessment must be based on immunoassays. For several HMW occupational [41] and domestic [38] allergens ad-hoc proposals have been made. However, these are of limited value, because the assays that are being used have not yet been rigorously standardized. The allergen concentration may vary according to the extraction method of the dust, the elution buffer, and the allergen standard used [35••,36,50]. Results also depend on the type of assay and also the antibody source used [51••]. Whereas these problems may potentially slow down progress in the epidemiological and risk assessment research field, they may also frustrate practical hygiene studies in the field, because study results cannot be compared when different assays have been used and no comparison has been published.

Conclusion

The use of immunoassays for exposure characterization of HMW sensitizers has led to major developments such as the possibility of studying exposure–response relationships in epidemiological studies. Applications to the standard setting are still limited and require rigorous standardization, but can be expected in the near future.

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