Exposure assessment of high molecular weight sensitisers: contribution to occupational epidemiology and disease prevention

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Abstract

An important group of sensitising agents are so called high molecular weight sensitisers—proteins or glycoproteins with molecular weights in the 5–70 kDa range that can provoke a specific IgE response in workers exposed to these agents. Exposure to high molecular weight sensitisers could only be evaluated indirectly in the recent past. Few measurement techniques existed that made it possible to measure the allergens directly. As a result, few studies focused on establishing exposure-response relations, and exposure standards have not been established for high molecular weight sensitisers, or those that have are of doubtful scientific basis. Recent use of immunoassays changed this perspective dramatically. Antibodies used in the assays can originate from human serum (sensitised workers), serum from sensitised animals (rabbits producing polyclonal antibodies), or animal derived cell cultures producing monoclonal antibodies. Although few comparative studies exist, the available evidence suggests that although the correlation of allergen concentrations obtained with different assays is good, large systematic differences occur. The use of conversion factors to make data from previously performed allergen measurements comparable or exchangeable is limited and thorough standardisation of assays is preferred. Validation and comparison of different assays by comparisons between laboratories seem important issues that have not received the attention needed. Epidemiological studies in several industries that used immunoassay for the exposure characterisation have shown that risk of sensitisation increases with increasing exposure to allergens. Several studies have also shown that clear differences in potency seem to exist. Sensitisation to rat urinary allergens and fungal α-amylase occurred in the pg/m^3 and ng/m^3 range. The main research questions of the near future have to focus on the prevention of occupational sensitisation. Standard setting seems possible for some allergenson the basis of the available scientific evidence for the existence of exposure response relations. However, assays for characterising exposure to allergens have to be rigorously standardised before they can be used under field conditions.

(*Occup Environ Med* 1999;**56**:735–741)

Keywords: assays; standards; high molecular weight sensitisers

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Accepted 1 July 1999

Asthma is one of the most common causes of chronic ill health. It is generally accepted that asthma is predominantly an environmental disease.[1] Sensitisation 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 sensitising agents show prevalences of an order of magnitude of 5%–50%.[2–12]

An important group of sensitising agents are the so called high molecular weight sensitisers, proteins or glycoproteins that can provoke a specific IgE response in workers exposed to these agents. Molecular weights are typically in the 5–70 kDa range. Several agricultural products and animal excreta contain high molecular weight sensitisers. Well known high molecular weight sensitisers are wheat (*Triticum*) proteins, rat and mouse urinary 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,[13–16] 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.[17 18]

Until recently, few measurement techniques existed to measure the allergens directly. In some of the early measurement series, from epidemiological studies, exposure to wheat has been assessed by traditional total dust measurements. This is not possible for potent allergens that sensitise workers at exposures in the ng/m^3 range. Exposure to latex allergen has in some instances been evaluated by measuring the protein content of the dust.[19] Enzyme exposure could only be evaluated with functional assays that measured enzyme activity through substrate conversion, but did not measure the allergen itself.[20] 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 sensitisation, were able to convert the same substrate. As a result, few studies focused on establishing exposure-response relations and therefore exposure standards have not been established, or, as the one for subtilisin from *Bacillus subtilis*, have a doubtful scientific basis because health

effects have been found with exposures below the level of the standard.[20]

The introduction of specific and highly sensitive immunoassays to measure the allergen content of dust samples has changed this perspective dramatically. Over the past decade epidemiological studies have been undertaken to study the relation between work related exposure to high molecular weight sensitisers and allergy. These studies were undertaken in bakery workers, laboratory animal workers, and enzyme workers. In these studies immunological techniques have been applied to assess levels of exposure to allergens. As a consequence, control strategies and standard setting based on scientific evidence are possible.

Immunoassays for assessment of exposure to allergens

Immunochemical methods use antibodies which are specifically directed against antigens that should be measured. These antibodies form measurable antigen-antibody complexes with the antigen of interest present in dust samples. Immunoassays can use isotopes, enzymes, and fluorescent or luminescent compounds as labels. Most immunoassays for the measurement of allergens are either inhibition or sandwich assays. Both are solid phase assays in which formation of antigen-antibody complexes occurs at a surface coated with a known amount of well characterised antibody or antigen. The binding of its specific counterpart from a fluid phase is measured by subsequent binding of another, so called "detecting" antibody labelled with—for example, radioisotopes, enzymes, or a fluorescent or luminescent agent. Especially, enzyme labelled assays (ELISAs), in combination with chromogenic substrates are commonly used and details on the ELISA techniques and other solid phase immunoassays can be found in textbooks.[21] In sandwich assays the allergen to be measured is captured between the antibody-coated surface and the detecting antibody which then is also joined to the allergen itself. In inhibition assays the concentration of allergen in for example, a dust extract, is measured as the capability to inhibit the binding of antiallergen antibodies to an allergen coated surface. A very important feature of both approaches is that the activity of a tiny amount of allergen can be measured without interference by many other, non-specific agents that are usually present in dust samples. This, however, has to be thoroughly checked as part of validation studies for each new immunoassay, and strongly depends on sensitivity and particularly specificity of the antibodies used. Specificity of antibodies as well as the properties and purity of calibration standards or other reference preparations can be assessed by, for example, gel electrophoresis and immunoblotting, which have also been used to compare reagents in analogous assay procedures from different laboratories. Sensitivity of inhibition assays depends mainly on the avidity and on the concentration of the inhibited antibodies in the assay. With high avidity antibodies (K_a values of 10^9-$10^{10}M^{-1}$) a sensitivity of 10–20 ng/ml for protein allergen molecules of 10–20 kDa can be reached. Sandwich assays can be much more sensitive, depending on the quality of the reagents; if sufficiently specific, the detection system can be considerably amplified by various secondary reagents, and in some assays sensitivities in the pg/ml range are possible.

Dust sampling

To measure antigens or allergens, dust samples can be taken with standard sampling equipment. Air is pulled at a constant flow rate through a filter mounted in a sampling head. The sampling head is designed in such a way that a specific size fraction of dust particles is sampled. For studies on occupational asthma and rhinitis it is common practice to sample the inhalable particles; particles that can penetrate the respiratory organs.[22] Hardly any information has been published on optimal filter material. Polytetrafluorethylene (PTFE or Teflon) and common glass fibre filters are the most commonly used filter types but validation studies are needed to support this choice. After sampling the allergen in the dust is recovered from the filter in water or a buffer solution. Like choice of filter material, optimisation of extraction procedures has been considered in only a few studies so far.[23–25] From a biochemical and physiological point of view it seems reasonable to use solutions with physiological pH and ionic strength, such as phosphate, Tris, or borate buffered saline. The addition of a mild detergent, such as Tween-20 or Tween-80 may improve the whole procedure by enhancing recovery during elution and preventing losses of small amounts of proteins sticking to filter material, pipette tips, tubes, and vial walls. Presumably, the optimal conditions for extraction may differ for each particular allergen, and may also depend on the type of samples from which the allergen is to be recovered.

Antibodies

Antibodies in the immunoassays can be specific IgE antibodies from sensitised workers, polyclonal antibodies isolated from serum of animals—for example, rabbits—immunised with the occupational allergen, or monoclonal antibodies produced by hybridomas made with spleen cells of immunised animals—usually mice or rats. Specific human IgE is theoretically the ideal antibody, as it by definition detects just the allergen to be measured, but is usually available in only limited amounts, as absolute concentrations of specific IgE are very low, even in severely sensitised people. Alternatively, specific human IgG antibodies might be used, which are often found in much higher concentrations, and in more exposed workers. The use of antibodies of the IgG_4 class may be specifically recommended, as many IgE inducing allergen molecules are also strong inducers of IgG_4 responses, as has been shown for several common allergens from, for example, pets, mites,[26] and wheat proteins,[27] and IgG_4 responses have been proposed to be specifically indicative of chronic inhalatory exposure as at the workplace.[28 29] Serum of several workers can be pooled to obtain an assessment of the total antigen or allergen

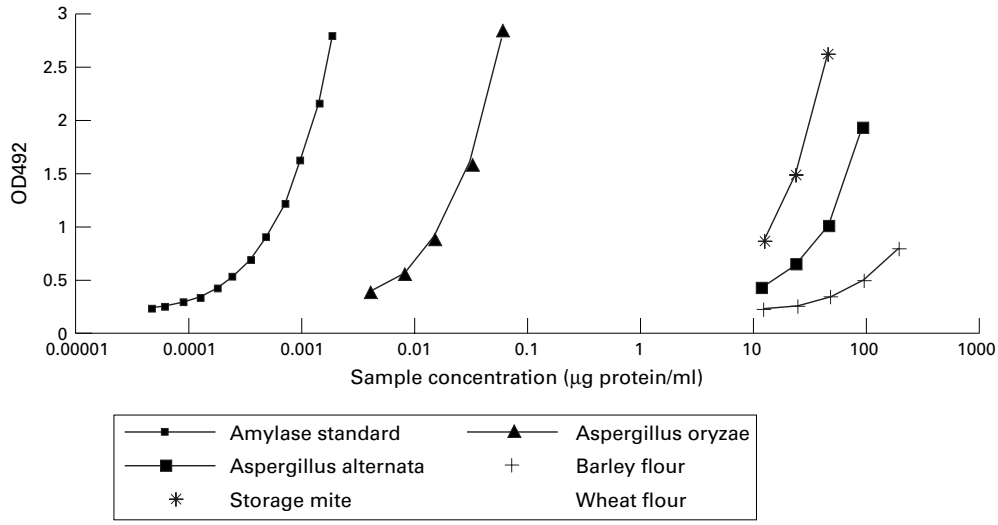

Figure 1 Specificity of the α-amylase assay as described by Houba et al[30] as shown by a series of inhibition curves for agents also present in the baking environment (OD492=optical density at 492 nm).

concentration. As the reaction profile of individual serum samples with a complex mixture of antigens might show marked differences, antigen measurements should be performed with pooled serum samples from at least 5–10 sensitised workers to prevent the exclusive or preferential measurement of only one or a few of the relevant components. Use of human IgG requires validation studies to show that the IgG has the same or very similar specificity as the IgE antibodies in sensitised workers. The use of antibodies of the IgG_4 subclass may therefore be specifically recommended, as IgG_4 and IgE responses usually show a very similar pattern of specificity, and IgG4 responses seem to be preferentially induced by chronic respiratory exposure. Similarly, validation of assays that use polyclonal or monoclonal animal antibodies includes a comparison of the specificity of these antibodies with IgE of sensitised workers. Production of antiserum may be time consuming, but, if successful, results in large amounts of immune reagents with high titres. Monoclonal antibodies have the obvious additional advantage of being highly specific, but their development is even more time consuming and requires specific laboratory facilities for production and maintenance of hybridoma cultures.

Allergen standards

When no purified allergen is available it is necessary to choose an allergen extract as a working standard. In many cases, potentially more than one allergen is present in complex dust mixtures such as grain dust, or dust from recycling plants. The allergen extracts should then be prepared from dusts from the industrial environment (department or working area) most likely to contain the allergen of interest. The allergen concentration can be expressed in relative units (equivalents or EQs) or in mass units of the allergen extract or protein content of the allergen extract. However, results cannot be compared between studies when different standards have been used. Sometimes only one protein is involved—for example, when bakery workers are exposed to α-amylase—in which case the purified protein can be used as a working standard. This allows the allergen concentration to be expressed more easily in comparable mass units.

A typical example is the assay developed to measure fungal α-amylase.[30] Fungal amylase allergens were measured with a sandwich ELISA, with affinity purified polyclonal rabbit antibodies (detection limit 250 pg/m^3). The reaction profiles of rabbit antibodies were tested with western blotting, and compared with the reaction profile of serum samples from IgE sensitised bakers. Rabbits and humans reacted to similar proteins, showing the validity of both assays. Furthermore, the test seemed to be specific and sensitive. Figure 1 shows some inhibition curves when the fungal α-amylase assay is tested with some potential constituents of dust from the baking environment. The curves show that inhibition is strongest for fungal α-amylase and only occurs for some other fungal allergen extracts. Even then inhibition only occurs at dilutions of another order of magnitude. On the basis of these results it would be expected that constituents of the dust other than fungal α-amylase will not influence the outcomes of the assay under ordinary conditions in the baking industry and will therefore not lead to false positive results.

Comparability of assay results

Few studies have been published on the comparability of immunoassays for measuring allergen concentrations in the air.[31–34] The most detailed comparison has been made as part of a collaborative European project (table 1). Methods to measure rat and mouse urinary aeroallergens at three institutes were compared. In total 222 (3×74 parallel) ambient air inhalable dust samples were taken from animal units in the Netherlands, United Kingdom, and Sweden (table 1).[31 32] After elution the extracts were analysed for rat and mouse urinary allergens. Median rat allergen concentrations obtained with a competitive inhibition radioimmunoassay (RIA) method were (a factor 3000 and 1700 times) higher than the con-

Table 1 Median (range) concentrations of rat and mouse urinary allergen (RUA and MUA) (ng/m³) found in ambient air dust samples taken in animal laboratories of the three participating countries (National Heart and Lung Institute (NHLI), United Kingdom; Wageningen University (WU), The Netherlands; National Institute of Working Life (NIWL), Sweden)

	Filters taken in								
	United Kingdom			The Netherlands			Sweden		
Method	n	Median (ng/m³)	Range	n	Median (ng/m³)	Range	n	Median (ng/m³)	Range
RUA									
NHLI	13	11000	172–52900	35	3730	<10.9–47200	25	775	<10.9–21700
WU	13	0.37	<0.16–15.0	35	0.86	<0.16–31.9	25	<0.16	<0.16–3.6
NIWL	14	1.95	<0.11–11.8	35	2.0	<0.11–43.4	25	0.71	<0.11–11.6
MUA									
NHLI	10	9.92	0.89–162	21	11.0	0.8–4610	20	9.37	0.74–82.5
WU	13	<0.16	<0.16–32.6	34	1.1	<0.16–1560	25	<0.16	<0.16–3.0
NIWL	14	0.24	<0.11–71.5	35	2.8	0.13–446	25	0.36	<0.11–6.1

The concentrations are stratified by the institute analysing the filters.[31]

centrations measured by enzyme immunoassay (EIA) sandwich methods. The difference between the two EIA sandwich methods was considerably smaller, by a factor of 2.2. The differences were smaller for the mouse allergen concentrations: factors of 4.6, 5.9, and 1.6 respectively. Addition of Tween to the elution buffer and antibodies used (monoclonal *v* polyclonal), were identified as the two main factors that caused the observed differences between sandwich assays. So, part of the differences are explained by differences between the methods, but variations in antibody specificity or composition of allergenic epitopes in the samples of air may contribute as well as the standards used (purified allergens, crude extract). Despite the systematic differences between assays, the correlation of allergen concentrations obtained by different assays seems very high.[31–34] The use of conversion factors to make data from previously performed allergen measurements comparable or exchangeable is limited and thorough standardisation of assays is preferred. Validation and comparison of different assays by comparisons between laboratories seem important issues that have not received the attention needed. The optimal situation to analyse allergens immunochemically is reached when the allergen has been identified, and purified allergen and monoclonal or polyclonal antibodies against the allergen are available.

Immunoassays in descriptive hygiene studies

Since the introduction of immunochemical techniques, a considerable number of exposure studies have been performed in a wide range of settings. Examples exist for evaluation of allergen exposure to enzymes such as papain in the meat processing industry,[35] fungal α-amylase in the baking industry,[30] exposure to egg protein,[36] pig and cow urinary and dander proteins in agriculture,[37 38] wheat allergens,[16 27] and rat and mouse urinary allergens.[39] The technique has also been used to evaluate allergenicity of fractions with different particle sizes. Studies in bakeries showed that especially larger particles contain allergenic wheat proteins and fungal α-amylase.[30 37]

Studies in the baking industry show that immunoassays are useful in characterising the exposure to wheat flour and α-amylase allergens in personal dust samples.[10 27 30 41–44] For several occupational titles clear differences in exposure to airborne allergens existed in these studies, where no differences in concentrations of dust could be found. These studies showed that the correlation between concentrations of dust and wheat allergen is moderate[43] and poor for fungal α-amylase.[30] Nieuwenhuijsen *et al*[10] reported correlation coefficients for dust and flour, dust and fungal α-amylase, and flour and fungal α-amylase aeroallergens of 0.65, 0.42, and 0.47 respectively.

The relation between dust and exposure to (wheat and α-amylase) allergens has been shown to vary considerably, depending on the job title, the size of the bakery, and the type of product produced by the bakery.[27] These findings are important as they allow inferences about the allergenic potency of dust in particular segments of the baking industry. For instance, there seems to be some debate about differences in the risk of sensitisation to wheat and fungal α-amylase in bread versus cake baking industries.[45] There are observed differences in sensitisation rates between workers in bread (n=392) and cake baking (n=77)—for example, wheat flour (6% *v* 3%), soy flour (7% *v* 1%), rice flour (4% *v* 1%) and fungal amylase (16% *v* 1%). Although the differences were not significant, these data suggest that bread bakers have a higher risk of sensitisation despite their presumably lower average dust exposure. However, such differences in risk of sensitisation found in a cross sectional study can also be explained by other factors, the interpretation of the definition of exposure (workers with regular exposure), differencesin duration of exposure, labour turnover between different sectors in the baking industry, and representativeness of the exposure measurements. Allergen measurements in dust would really allow direct inferences about the reasons for the apparent difference in risk.

Concentrations of wheat allergen can to some extent be predicted on the basis of information on dust concentrations, products made, and the process used. However, reliable prediction would require many dust measurements, and measurement of the allergen in the dust by immunoassays seems more efficient.[42] The results of these analyses suggest that allergen concentrations reflect true exposure more accurately then crude surrogates such as dust

Table 2 Relations between sensitisation and exposure to rat urinary allergen (mean rat urinary allergen concentration in ng EQ/m³ multiplied by the number of hours exposed per week), sex, and smoking for atopic and non-atopic subjects in a cross sectional study in 650 laboratory animal workers[5]

	Non-atopic subjects		*Atopic subjects*	
	PR	*95% CI*	*PR*	*95% CI*
No exposure	1.0	—	1.0	—
>0–0.5 ngEQ/m³×h/week	1.5	0.2 to 8.8	3.1	1.2 to 8.0
>0.5 to 8 ngEQ/m³×h/week	3.1	0.8 to 12.4	3.1	1.2 to 7.8
>8 ngEQ/m³×h/week	4.4	1.1 to 17.1	4.2	1.6 to 11.2
Sex (f *v* m)	0.6	0.3 to 1.6	1.1	0.6 to 2.0
Smoker (smoker *v* non-smoker)	1.3	0.5 to 3.6	0.9	0.4 to 1.8
Ex-smoker (ex-smoker *v* non-smoker)	1.0	0.2 to 4.8	0.8	0.3 to 2.0

PR=prevalence ratio; 95% CI=95% confidence interval.

exposure. Similar experiences exist with measuring other high molecular weight sensitisers.[46–49] Interestingly, dust concentrations in laboratory animal units are extremely low and useful exposure characterisation is only possible by measuring the allergens directly, as dust concentrations in many animal rooms will be below the detectionlimit.

Use of immunoassays in exposure-response studies

Risk of sensitisation has been shown to increase with increasing exposure to aeroallergens in cross sectional studies in several industries.[3–8] For example, the risk of developing laboratory animal allergy has been found to be associated with the concentration of allergen. In these studies exposure to airborne allergens from laboratory animals was measured with sensitive immunoassays. Data from three independent studies among laboratory workers have been pooled into a large cross sectional study as part of a European collaborative project (table 2).[5] The data were from three cross sectional studies in The Netherlands, the United Kingdom, and Sweden and involved 1062 laboratory animal workers. Selection criteria were harmonised, and this resulted in a study population of 650 laboratory animal workers (60.5% women) with <4 years of exposure. Air allergen concentrations were assessed previously in each country and converted arbitrarily to Dutch allergen concentrations on the basis of the allergen analysis comparison between laboratories already described.[31 32] Available serum samples 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 a week with rats was more strongly associated with sensitisation than mean exposure or the number of hours worked with rats alone.

The sensitisation rate increased with increasing exposure to air allergens. Interestingly, this study suggests that there is an increased risk of sensitisation at very low levels of exposure. Atopic workers exposed for only a few hours a week to low exposure concentrations of 0–0.5 ngEQ/m³.hours/week (exposure category arithmetic mean exposure 0.18 ngEQ/m³.hours/week) had more than a threefold likelihood of being sensitised than non-exposed workers. Atopic workers in the highest exposure category with exposure concentrations >8 ngEQ/m³.hours/week had an almost fourfold increased risk of sensitisation, but their mean exposure was >1000 times higher than for the category with least exposure (exposure category arithmetic mean exposure 188 ngEQ/m³.hours/week). Thus, for atopic workers there was little increased risk with increasing exposure, whereas for non-atopic workers a steadily increasing risk was found (table 2). The results suggest that the lowest exposures observed seem sufficient to sensitise a considerable proportion of the atopic workers, whereas the risk of becoming sensitised for non-atopic workers at these concentrations is almost negligible and becomes noticeable only at higher levels of exposure.

These results are indicative of an extremely steep exposure-sensitisation curve for rat urinary allergens, which suggests that a possible exposure standard will need to be extremely low and that exposure control needs to be extremely rigorous to minimise risk of sensitisation.

The available studies to date also allow comparisons to be made between the various exposure-response relations for different allergens and occupational populations. A tentative analysis suggests that there is a wide range in sensitisation potency between various allergens (fig 2). The allergen exposure in these studies has been evaluated by one laboratory, and results have been corrected for the protein content of the allergen standard. Sensitisation against rat urinary proteins occurs in the pg/m³ range.[5] Fungal α-amylase sensitises in the low ng/m³ range as shown in two cross sectional studies of bakery workers 178 and 256,[7 10] whereas sensitisation against wheat flour occurs in the μg/m³ range as found in a cross sectional study among 392 bakery workers.[8] Sensitisation against animal proteins from pigs and cows suggests exposure concentrations also in the μg/m³ range.[37 38] Criteria for sensitisation differ between studies but a change in the criteria will only affect the slope of the exposure-response relation, not the position on the exposure axis. The differences found cannot be attributed to differences in sampling or allergen extraction as analyses were performed in one laboratory. The differences are also too large to be explained by the number of allergens present in dust samples. Commercial fungal α-amylase preparations and rat urinary proteins contain between one and a few major allergens. Wheat, on the contrary, has been shown to contain more than 40 different water soluble antigens. However, the difference in potency is more than 10-fold and the difference remains even if corrected for the potential number of allergens in the dust. The limited potency of wheat flour explains to some extent why the correlation between exposure to dust and allergen concentration is reasonable. For some of the extremely potent allergens, dust concentrations cannot be used to approximate the allergen concentrations in the air.

Results of these epidemiological studies show the potential improvement in results when valid exposure data are available. Further progress is expected when more complex

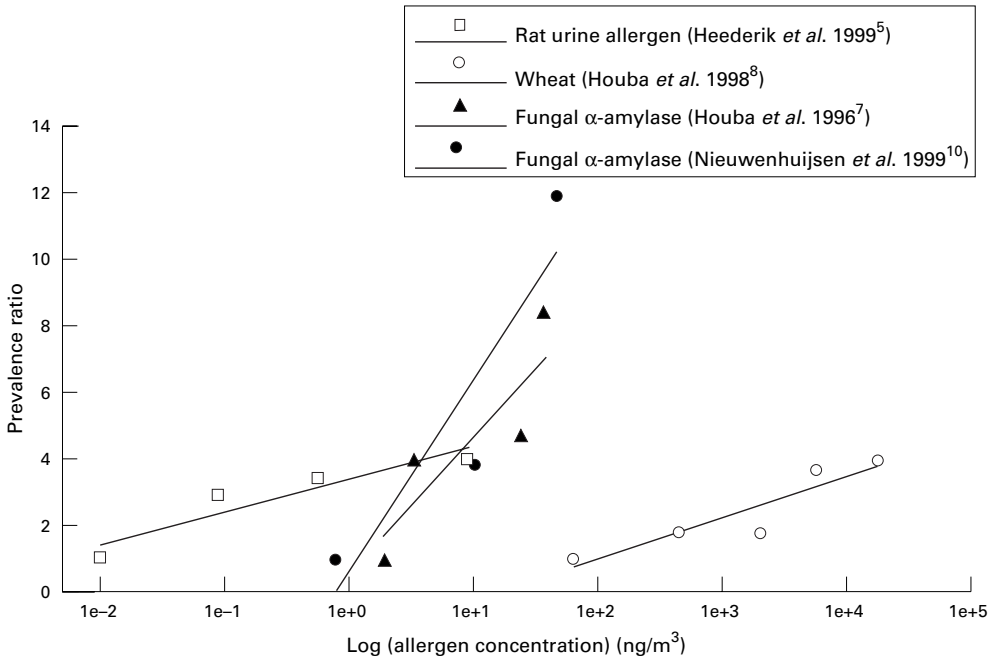

Figure 2 Exposure-response relations for sensitisation against rat urinary allergens (sensitisation defined as IgE>0.7 kU/l) (from table 2 mean exposures of 0, 0.09, 0.57, 8.8 ng/m³, Heederik et al[5]), wheat allergen exposure (sensitisation defined as IgE>0.35 kU/l, Houba et al[8]), and fungal α-amylase (sensitisation defined as IgE>0.35 kU/l (Houba et al[7]), and skin prick test wheal>3 mm (Nieuwenhuijsen et al[10]).

statistical methods are applied to evaluate the shape of exposure response curves, and available longitudinal data will be analysed. The main research questions of the future have to focus on the prevention of occupational sensitisation. Standard setting seems possible for some allergens on the basis of the available scientific evidence for the existence of exposure-response relations.[49] However, some issues remain to be solved. Evaluation of no observed effect levels is difficult as long as little is known about the shape of the exposure-response relation. It is also often not clear what normal sensitisation rates are for most allergens, which makes it difficult to evaluate what increase in sensitisation rate in occupationally exposed populations should be regarded as an adverse effect. Therefore, intervention studies are warranted to evaluate the effectiveness of standard setting and implementation of the resulting exposure control strategies.

Exposure control and prevention

Little quantitative information is available on the contribution of different determinants of exposure in industries with exposure to allergens (equipment, technology, production lay out).[50] Most studies were descriptive and aimed to monitor exposure levels for epidemiological purposes. Recently some hygiene studies have been conducted in the baking industry that describe the relation between exposure to dust and allergens, and some determinants of exposure.[41 42 51]

Several experimental studies have been undertaken in the past in laboratory animal units to evaluate the effect of certain control measures on concentrations of environmental allergens. Recently results from a larger observational study were published which showed that determinants of personal exposure were different from the determinants of environmental exposure.[52] Several observational studies confirmed that concentrations of rat aeroallergen are determined by differences between work sites, tasks performed, stock density, and the exposure group.[46–48 50] Most of the hygiene studies also showed that determinants of exposure to dust are not necessarily the same as for exposure to allergens. This again shows the use of allergen measurements versus conventional measures of exposure to dust when determinants of exposure have to be identified for exposure control studies. Exposure modelling also makes it possible to predict to some extent what the change in exposure will be when some determinants of exposure will be removed or otherwise influenced. For instance, the effect of changes in stock density and ventilation rate on exposure to rat urinary allergens has been described.[48]

Conclusions

Immunoassays have been used over the past decade in hygiene and epidemiological studies. Development of immunoassays for characterising exposure to allergens in the work environment opened new research avenues and contributed to improvement of epidemiological studies especially studies that focused on the evaluation of relations between exposure and sensitisation. Without immunoassays, exposure assessment of high molecular weight sensitisers would not have been possible. Identification of determinants of exposure and exposure-response studies would have to be based on exposure proxies that are less accurate than direct measurement of the allergens. Health based occupational exposure standards still do not exist for high molecular weight sensitisers, but development of exposure standards based on scientific evidence seems

now to be possible for some allergens—such as wheat proteins, rat urinary allergens, and fungal enzymes such as α-amylase. However, the number ofhigh molecular weight sensitisers is largeand only a few can presently be measured-with immunoassays. Moreover, standardisation of these assays is important beforethey can be widely used under field conditions.

Another advantage of immunoassays is that the efficiency of strategies to control exposure can be evaluated. In many situations measures to reduce exposure were proposed, that could not be evaluated directly by exposure measurements. Medical surveillance was needed as an indirect tool to evaluate risk before and after sensitisation after control measures were taken. Direct evaluation is faster, more efficient, and less costly, and especially when in combination with exposure-response data, is the strongest strategy available to control exposure.

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