Allergen and irritant exposure and exposure-response relationships

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Abstract

Exposure to allergens and irritants is a complex phenomenon. Especially the particulate nature of many natural allergens in combination with the low exposure creates remarkable phenomena. Many allergens appear to be potent sensitizers in the nanograms per cubic meter of air range. Evidence exists that workers can be sensitized even after exposure to a low number of particles. With exposure in the low nanogram range, the respiratory tract is exposed to distinct exposure quanta of a few particles, which lead to high local concentrations of allergenic molecules. With gaseous exposure, a more equal exposure over the large surface area of the respiratory organ is to be expected. These phenomena may have mechanistic and biological implications, but certainly determine exposure assessment approaches, the interpretation of exposure measurements and the evaluation of exposure-response relationships.

Introduction

Exposure-response relationships are considered important because they point towards options for prevention. Exposure is a complex phenomenon and varies considerably over time and space. Characterizing exposure for assessing relationships with asthma occurrence in epidemiological studies requires understanding of exposure phenomena. These phenomena in their turn determine the approach in the analysis to a large extent. When all this is taken in consideration, exposure-response relationships can be analyzed. Many examples exist of exposure-response relationships for low- (LMW) and high- (HMW) molecular-weight agents, mainly from industry-based studies in which the exposure has been measured in quantitative terms. Some of these examples are presented and discussed in this chapter. General population studies with less detailed exposure information are not discussed. Recent developments such as the use of exposure-response information in risk assessment is only briefly covered here.

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Allergens and irritants

An allergen is a non-parasitic/non-pathogenic/non-infectious antigen capable of inducing immune-mediated hypersensitivity reactions in sensitized hosts. The preceding 'sensitization' consists of allergen-specific immune responses to a previous exposure, upon which specific T cells and antibodies have been produced – as a rule, without any observable symptomatic adverse health effects. During secondary exposures of the sensitized host, the specific antibodies and/or T cells recruit and activate inflammatory cells and mechanisms that normally should protect against harmful pathogens. For normally harmless allergens like grass pollen, cat saliva or dog skin flakes, however, the costs of these inflammatory reactions – adverse health effects, and allergic symptoms – heavily outweigh the benefits of immune protection. "Atopic" or type I hypersensitivity refers to allergy based on specific IgE and so-called Th2-type sensitization. Since this is the best studied type of allergy associated with work-related respiratory disease, this chapter further focuses exclusively on type I allergens and IgE-mediated allergy.

Allergens causing type I-mediated ('atopic') immunological asthma can be both LMW and HMW sensitizers of which more than 250 have been identified. LMW sensitizers are often synthetic industrial chemicals such as isocyanates, acid anhydrides, metals and metal salts, but also natural substances such as plicatic acid from western red cedar wood. LMW sensitizers are supposed to react with and to bind covalently to human proteins in the respiratory tract mucosa, and to function as major epitopes in the thus-produced immunogenic 'hapten-carrier' complexes. The epitope specificity of the produced antibodies in the serum of the sensitized worker can be demonstrated by showing their binding in diagnostic tests with the LMW hapten (like isocyanate) coupled to a range of different carriers.

HMW sensitizers are naturally occurring water-soluble proteins in the 10–60-kDa molecular mass range that in a hydrophilic environment, like the respiratory mucosa, are readily released, e.g., from skin scales, plant fibers, pollen grains, and other tissue matrices. Some allergenic products contain only one or a few allergens. For instance, commercially graded fungal α-amylase contains the active enzyme, with a molecular mass between 51 and 54 kDa, and some other allergenic compounds of 25–27 kDa and 40 kDa, probably enzyme fragments or fungal products [1]. Wheat flour has been shown to contain more than 100 allergenic molecules, of which 40 have been identified [2]. For such complex products like wheat, latex, etc., the exposure assessment should either be based on measuring one single marker molecule, using highly specific monoclonal or polyclonal antibodies, or on characterizing and measuring the whole mixture of antigenic/allergenic proteins with a pool or mixture of polyclonal antibodies [3]. Both approaches have been used and so far have resulted in allergen assays with strongly concordant results [4, 5].

The situation may be analogous for some LMW sensitizers like isocyanates, where exposure occurs to a mixture of the monomer and monomer-derived oli-

gomers. The most recent studies have measured several monomers and oligomer molecules, and expressed exposure in total isocyanate concentration [6–8]. This is a simplified way of combining exposure to a range of molecules with probably different allergenic potency into one metric. Theoretically it would be possible to weigh the relative contribution of an individual molecule to a mixture by its allergenic potency. However, this information is only available for a limited number of isocyanate molecules tested in experimental animal studies [9, 10] and is not possible practically.

Irritants are agents for which the effects do not depend on preceding specific sensitization. They can thus provoke acute and transient narrowing of the airways at first exposure of an individual, and may do so through a variety of non-immunological mechanisms such as mast cell mediator release, and interaction with sensory nerve endings in bronchial epithelium or receptors in smooth muscle. Irritants may have stronger effects in individuals who are bronchially hyperresponsive. A common incited acute response to irritants should be distinguished from the induction of reactive airways dysfunction syndrome (RADS). While the same chemicals that incite transient airway narrowing can also cause RADS, induction of the latter seems the result of extremely high peak exposures to chemical irritants such as chlorine compounds, ammonia, diisocyanates, etc. Irritant exposure is of interest because it may also interact with allergen exposure in the sense that the risk for developing allergy and asthma may be modified. Examples are diesel exposure and sensitization to common allergens [11] and exposure to disinfectants in farming and atopy and bronchial hyperresponsiveness [12].

Exposure and exposure routes

Exposure is defined as contact between a target, in the context of this chapter a human, and a chemical, biological or physical agent in an environmental carrier medium [13]. For occupational asthma (OA) contact between the respiratory organ, and air as a carrier containing allergens or irritant gases or particulates, is the most relevant exposure. Recent indications suggest that dermal exposure might also play a role. Uptake of an agent is determined by the concentration in the medium (concentration in the air), the uptake of the medium (ventilation, inhalation), and clearance from the lung. The dose of an agent is the amount that enters the target, in this case the respiratory mucosa and underlying tissues. The amount that enters the upper and lower airways is, therefore, not always the relevant amount, because for instance a large fraction of very small particles – in the 10–100 nm range – and inert, non-soluble gases may also be directly exhaled, thus leading to a lower effective dose. So dose should be defined as the amount that is absorbed by (in case of gases), or deposited on (in case of particulates) the respiratory mucosa. One should consider that the definition of ‘dose’ may depend on the mechanism of the studied

health effect: whether it only requires interaction with cells and molecules in the mucosa, or also needs to migrate through the respiratory basal membrane to submucosal tissue, or even the surrounding capillaries or lymph nodes. It should be realized that most information about particle deposition is based on research with inert radiolabeled particulates. Very little is known about the role of deposition, clearance and retention of allergenic or irritant particulates. Particle deposition is size and shape dependent and differs for different regions of the respiratory organ. For near spherical particulates, behavior is well described by the aerodynamic diameter. The aerodynamic diameter is an expression of the aerodynamic behavior of a particle (for a perfect sphere with unit density, the diameter equals the aerodynamic diameter). Generally speaking, larger particulates deposit in the nasopharyngeal region (including nasopharynx, oral passages, and larynx) by sedimentation and impaction. Retention times in this region are short, usually between minutes to hours [14]. In the tracheo-bronchial tree, which includes the trachea, bronchi and bronchioli, particles are deposited by impaction in the upper part and by sedimentation in the lower part. Non-soluble particles are usually cleared within 1 day by the ciliated airways and most are swallowed. In the alveolar zone, particles deposit by sedimentation and diffusion, and are removed very slowly by cellular clearance mechanisms with clearance times from months to years. Different size fractions have been defined which can penetrate different regions of the respiratory organ [15]. For OA research, the inhalable dust fraction (50% cut-off at 10 μm) is the most important dust fraction, and is defined as those particles which can penetrate the human respiratory organ. Some literature exists that indicates that deposition in the nasal region can induce reactions distal from the nose, so larger particulates may be relevant for effects lower in the airways [16]. Traditionally, occupational hygiene studies focused on pneumoconiosis and monitoring programs commonly measured the fraction of respirable dust particles (50% cut-off at 4.25 μm) of which the majority can penetrate the alveolar region. This fraction is more relevant for toxicants for which uptake through the alveoli can take place, and for dusts which cause pneumoconiosis. These particles are smaller than most of those in the inhalable fraction. The respirable fraction underestimates the exposure to larger particulates for which deposition in both the upper and lower airways may also be of primary importance, like most of the known allergen-carrying particles. It should be further emphasized that the boundaries between the various particle fractions and the regions where these are deposited are not sharp. Thus, while the majority of allergen particles, e.g., of 10–20 μm diameter, will be deposited in the upper airways, a substantial proportions may reach the lower respiratory tract and induce not only rhinitis but also asthma symptoms.

Dermal exposure is a considerably less explored field in exposure assessment for OA. There is clear animal evidence showing that LMW sensitizers, like isocyanates, may induce sensitization after dermal exposure, with subsequent inhalation challenges resulting in asthma-like responses [17]. Several lines of evidence support a

similar role for human isocyanate skin exposure, namely, that dermal exposure may contribute to the development of isocyanate asthma by inducing systemic sensitization [18]. More research is needed in this field to develop and improve dermal-exposure assessment methods for sensitizing agents [19, 20], since objective and reproducible methods are lacking and it is unclear how dermal exposure should be measured in a biologically relevant way for OA. Dermal exposure is usually measured by hand washing methods, dermal patches, or by analyzing gloves, but none of these approaches are completely satisfactory.

Variability of exposure and exposure patterns

Temporal aspects of exposure are considered important, e.g., is the exposure relatively constant and at the same level, or are there fluctuations or possibly even sharp and large increases over time (peaks)? Many allergen exposed workers are exposed to a pattern of high peaks over a working day. Measurements with continuously registering devices have shown that bakers are exposed to peaks of flour or enzymes when they empty bags, dust dough, or clean the bakery [21]. These peaks occur when flour particles become airborne because of physical forces, but since they are relatively large (aerodynamic diameter 5–15 μm and often even larger [22, 23]) they will reside in the air for only a brief period of time. As a result, even the highest peaks last for a maximum of only several minutes [21], and between these task-related high peaks the exposure will be very low. This exposure pattern is typical for many situations with exposure to HMW and non-gaseous LMW sensitizers, because relatively large particulates are involved, and peak exposures are usually the consequence of regularly performed daily tasks. Very high dust and allergen exposures, however, may also occur infrequently, e.g., due to repair or cleaning activities of damaged or neglected equipment or storage sites. Examples are environments such as laboratory animal facilities with exposure to rat and mouse urinary proteins, the farm environment with exposure to allergens and microbial agents such as endotoxins, health care settings with exposure to latex. The baker's work is typically cyclic, with a daily repeated pattern of exposure peaks, and the average exposure over the day is therefore relatively similar from day to day. Thus, within a day the exposure is highly variable because of the sequence of peaks and low background levels, but between days the differences are relatively small. Among laboratory animals workers day-to-day variation in exposure may be much larger. Typical high-exposure tasks are the handling of living animals and the cleaning of cages and removal of cage bedding. For workers routinely involved in cage cleaning there may be regular temporal patterns of high exposure, and also for animal caretakers there may be daily tasks, e.g., feeding, leading to more or less cyclic exposure patterns. Researchers performing animal experiments may, however, often have days or even weeks with practically no allergen exposure. Exposure assessment

in this job category therefore strongly relies on combinations of allergen measurements and careful monitoring – using diaries or questionnaires – of performed tasks. Allergen-exposure levels in farming have hardly been studied, but may *a priori* be expected to show a very large variation. In animal farming many job tasks show daily patterns – like feeding and milking – and on many days the average daily exposure may thus be relatively constant. Other potentially high-exposure activities, however, like emptying and cleaning of stables and barns, occur regularly but with much lower frequency over the year. In crop farming the exposure to plant or microbial allergens will be strongly associated typical season-related activities, like harvesting. Although no data on allergen exposure are available, results for airborne dust and endotoxin levels in farming and various agricultural industries suggest that it indeed shows very pronounced within-day but also day-to-day and seasonal variation [24, 25]. Similar patterns may also occur for LMW sensitizers such as diisocyanates used in spray painting. In industrial spray painting, the within-day variation may be high but daily exposure patterns relatively constant, whereas in many small companies – notably car repair shops – the day-to-day variation may also be very large depending on the actual work available. Apart from that, there may be other reasons for incidental peak exposure. Spray painters usually work in highly controlled environments such as spray booths with exhaust ventilation systems. As a result, the exposure is often below the limit of detection but high exposures occur because they regularly spray outside the booth for small repairs or during formulation of paint, cleaning of equipment and because of spills [6].

Physical and chemical aspects of airborne allergen exposure

It is useful to conceptualize how exposure to allergens occurs. Large measurement series in the baking industry, as part of an exposure-response study on fungal α-amylase [26], showed that workers with a high exposure were exposed to time-weighted average levels of fungal α-amylase between 5 and as high as 100 ng/m^3. These data were obtained with full-shift (8 hour) measurements, and thus represent daily averages. Moderately exposed individuals were exposed to levels between 0.5 and 5 ng/m^3, but in this category more than 70% of the measurements were below the limit of detection [26]. Workers in the high-exposure category were mainly dough makers working with pure enzyme formulations. Workers with "moderate" exposure levels did not handle pure enzyme but batches of cereal flours to which the enzyme had been added – either elsewhere in the bakery or in the flour-supplying industry. Since amylase is added in only milligrams quantities per kilogram flour (a 1/10^6 ratio on the basis of weight), the amylase exposure at a worksite between 1 and 5 mg/m^3 flour dust exposure – common for dough makers – would be around 1–5 ng/m^3, which agrees well with the observed average amylase levels. It is, however, less easy to understand why for the majority of measurements in this exposure

category the amylase levels remained under the limit of detection. A likely explanation might be the particulate nature of the amylase, which is added as solid powder to the cereal flours, with as consequence a highly heterodisperse distribution of airborne amylase. Since these allergen exposure characteristics may have important consequences for exposure-assessment strategies, and for the interpretation of exposure-sensitization relations, they are discussed below in some more detail. Both wheat and airborne amylase allergens have been mainly found in airborne particles with aerodynamic diameter between 5 and 15 μm [22, 26]. Assuming a spherical shape and density of 1–1.5 g/cm^3, one may estimate their mass to range between 0.04 and 2 ng. Thus, if amylase is added to flours as a 50–100% pure enzyme powder, each milligram of the resulting 'mixture' consists of approximately 1–10 million wheat flour, but only 1–10 amylase particles; when the mixture becomes airborne, a moderate dust exposure of 1 mg/m^3 would mean that workers will be exposed to a very limited number of amylase particles per cubic meter. Due to the random particle distribution at such low numbers, however, airborne measurements – with usually close to 1 m^3 air sampled per filter (on the basis of a sampler sampling 2–3 l/min) for personal full-shift air samples – will inevitably show a high coefficient of variation (CV) and potentially many samples with an allergen content below the detection limit. In fact, with on average only a handful of particles per air sample, there is a substantial risk of having no amylase particle at all in a sample, and this may be one of the reasons that even at moderate average levels many samples remain negative when tested for the presence of amylase.

The heterodisperse nature of some airborne allergens can be confirmed by direct staining methods, like the HALOgen procedure [27] in which allergen-specific antibodies are used to visualize the allergen molecules released from a particle trapped on a filter or adhesive tape. Application on personal inhalable dust sample from baker's work environment clearly confirmed that only very few of the many dust particles on a filter showed the presence of α-amylase (Fig. 1).

The airborne dust composition described here may be exceptional, with all allergenic (amylase) activity concentrated in a few particles per cubic meter, surrounded by ~10^6 other non-allergenic particles – or particles with other allergenic specificity like wheat proteins in this particular case. For 100% pure enzyme powder the number of allergen molecules per particle is, given a molecular mass of around 52 kDa for fungal α-amylase, extremely high and varies between 10^9 and 10^{10} per particle, depending on its exact size. So amylase exposure appears to occur in the form of a limited number of 'allergen quanta' consisting of particles with a large number of allergenic molecules per particle. For other allergens the situation might be less extreme, but the same principles may be applied, e.g., to animal dander particles, or fecal particles from house or storage mites. Although the latter contain many other molecules, it has been shown that each mite fecal particle may contain up to 2.5–5% (w/w) of the major allergen Der p1, which, assuming a particle diameter of approximately 20 μm and a molecular mass of 24 000, implicates 'allergen quanta'

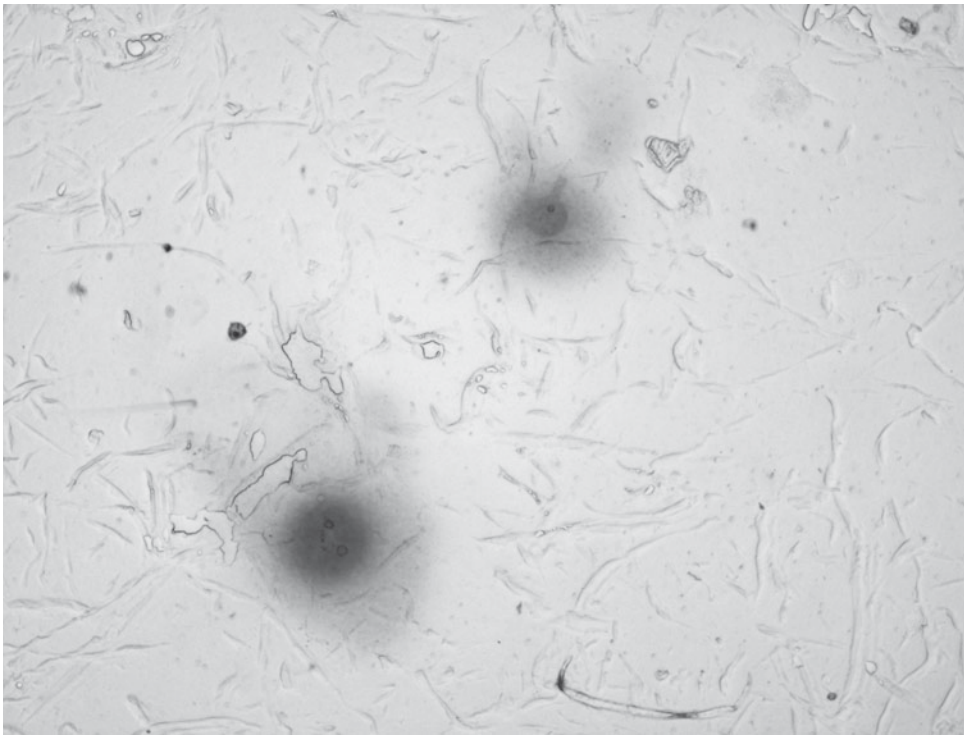

Figure 1.
HALOgen staining of α-amylase in an airborne bakery dust sample (courtesy J Bogdanovic, IRAS UU). The sample shown was taken during a 30-minute period when a bakery worker was dusting dough with enriched flour. Two amylase containing particulates are visible. The whole filter contains not more than 25 particulates, indicating that a bakery worker, handling enriched flour may be exposed to a very low number of amylase containing particulates per day.

of no less than 10^8–10^9 Der p1 molecules per particle [28]. Parallels also exist for exposure to pollen. Grass pollen exposure ranges from a few to several hundreds of pollen per cubic meter measured as long-term average concentration [29, 30]. When exposed to water, pollen grains may rupture at the single germinal aperture, releasing around 700 granules of less than 3 μm in diameter from each grain [31]. This leads to several hundreds to thousands of particulates per air samples [29, 30, 32], but still a relatively low number considering the number of allergen molecules involved.

This is in clear contrast to a spray painter working with a diisocyanate monomer or a monomer oligomer mixture of, for instance, 1,6-hexamethylene diisocyanate

(HDI). Monomer exposure will to a large extent be gaseous because of the vapor pressure of HDI. This implies that even at low exposure levels, a large number of molecules will be inhaled by, and dispersed across, the respiratory organ. Diisocyanate exposure to oligomers of HDI, however, occurs in the form of small aerosol droplets, likely with limited number of particulates containing many allergenic molecules with free isocyanate groups. There are indications from experimental animal studies that gaseous monomer exposure is more potent at eliciting respiratory responses than particulate exposure, and that differences in particle size distribution are also associated with a quantitatively different response [10, 33]. Many of these differences may be due to a different deposition pattern for different particle size distributions, leading to differences in dose in specific zones of the respiratory organ. For mixed particulate and vapor exposure, the vapor molecules may be adsorbed to the surface of the particle and penetrate deeper into the airways and lungs than in the case of gaseous exposure only. This may also contribute to a qualitatively and quantitatively different response.

It is not clear what the biological implications of localized high exposure are. It may be important to discuss the question separately for the allergic sensitization process and for the induction of symptoms in sensitized subjects. What distribution and what levels are sufficient to lead to a high sensitization risk? Results from dermal sensitization studies suggest that a few antigen-presenting dendritic cells encountering many molecules may induce a more vigorous response than many dendritic cells encountering only a few molecules [17]. Thus, the focal presentation of a very small number of allergenic molecules concentrated on a few cells may represent an exposure pattern that results in a major risk for sensitization. It therefore seems important to consider the nature of the exposure pattern in terms of aggregation phase (gaseous, particulate), particle distribution, and time pattern of exposure (peaks) [34]. Incidental (peak) exposure to a very limited number of particulates seems associated with only a mildly elevated risk for sensitization risk [26, 35]. Specific IgE titers against fungal α-amylase appeared to be low in workers with moderate exposures to fungal amylase [26]. However, workers involved in handling pure enzyme can be exposed more frequently to short exposure peaks of up to a few hundred milligram per cubic meter of pure enzyme for a few minutes [21]. Over a working day this will average out to a lower level, in the $\mu g/m^3$ range, and for longer periods – weeks to months – the average levels may be far below 1 $\mu g/m^3$. Thus, the increased sensitization risk observed at average exposure levels in the ng/m^3 range may in fact be the result of incidental 'sensitizing events' when the exposure increases suddenly but very briefly to 100–1000-fold higher levels.

The phenomena described above may also play a role in LMW sensitizer exposure. Spray painters are now more often exposed to oligomers than simply just gaseous monomers. Oligomer exposure is more likely to occur in the form of particulates. Exposure is very often below the limit of detection and is highly variable when above this limit and differs from moment to moment by more than a factor

10–1000. Spray painters often seem to face very low exposure, but during intensive spraying, or mixing of paint, brief periods of high exposure occur [6].

Exposure-response relationships

Studying exposure-response relationships for asthma is complex. The manifestation of the disease is variable and this complicates measurement of the presence of disease. The threshold concentration for elicitation of an allergic airway reaction in sensitized individuals is generally assumed to be lower than the threshold concentration to induce sensitization [17, 36]. Direct evidence for this observation is limited because elicitation levels for symptoms and other signs are poorly documented both in experimental and observational studies. It is only recently that allergen exposure data have been applied for exposure-response modeling using observational data from human populations. Some sensitizers, especially LMW ones like diisocyanates and acid anhydrides, may also have irritant properties. Irritating effects may occur in parallel at lower levels in atopic individuals who have been sensitized to common allergens, but not sensitized to the occupational allergen. Thus, although the evidence is not always very strong, different exposure-response relationships may be anticipated for different subgroups of workers. Theoretically, the slope and intercept of the exposure-response relationship might differ between individuals in the same workplace according to the mechanism of disease provocation (and therefore host factors) [36].

A clear definition of the actual health outcome studied is crucial for any statement regarding exposure-response relationships, and this is particularly true for the development of asthmatic disease. A simple model for the pathogenesis of OA induced by HMW sensitizers is usually represented as follows (Fig. 2): exposure leads to a series of events – sensitization, and development of an inflammatory response, which is accompanied by symptoms, bronchial hyperresponsiveness, airflow variability, etc. When exposure is continued, chronic changes and severe airflow impairment might occur. This process can develop rapidly and, as a result, it seems likely that workers may try to influence their exposure when they develop symptoms by migrating to jobs with lower exposure or leave the workforce (healthy worker effect). Examples of exposure-response relationships for the first step, sensitization, have been described for many allergens. For some LMW sensitizers exposure-response relationships have been observed. One prospective study included 163 previously unexposed workers with exposure to epoxy resins containing organic anhydrides: hexahydrophtalic anhydride (HHPA), methyl hexahydrophtalic anhydride (MHHPA), and methyltetrahydrophtalic anhydride (MTHPA) [38]. These workers were followed for, on average, 32 months (1–105 months). The levels of organic acid anhydrides in air and of specific IgE and IgG in serum were monitored repeatedly. The mean combined organic acid anhydride exposure was 15.4 $\mu g/m^3$

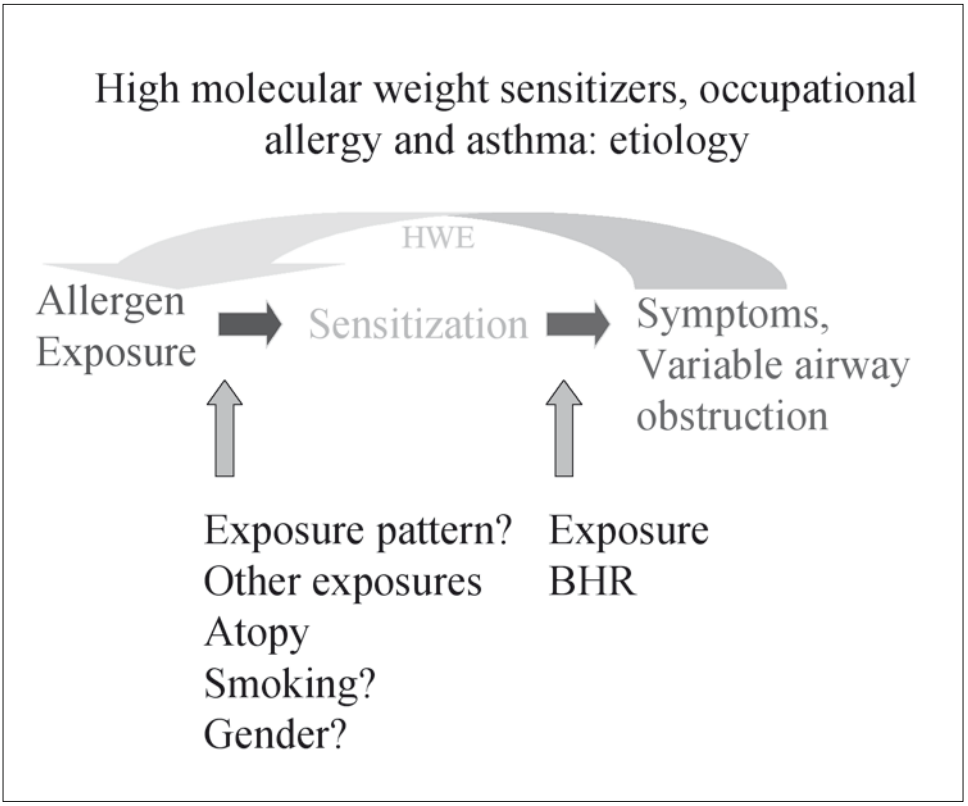

Figure 2.
Etiological model for the development of occupational asthma (after [37]).

(range < 1–189 μg/m^3). An exposure-response relationship was clearly demonstrated for the incidence of sensitization with increasing exposure. Specific IgE was demonstrated by 21 (13%) subjects with a mean induction time of 8.8 (1–35 months). The sensitization incidence was 4.1/1000 person months at risk. Atopics had a more than fivefold elevated sensitization risk compared to non-atopics.

Several other authors have also described exposure-response relationships for various organic acid anhydrides [39–41]. Similar observations have been found for some other LMW sensitizers such as platinum salts [42, 43] and isocyanates [44], although the available evidence is more limited than for organic acid anhydrides and the HMW sensitizers discussed in the following section. Early studies already gave some indications that exposure-response relationships could be observed for HMW sensitizers. Musk et al. [45] showed that bakery workers with a "high dust rank" were more often sensitized against one or more bakery allergens compared to work-

ers with a low dust rank. The dust classification was validated with total dust measurement. More recent studies in bakers used dust sampling instead of the – from an immunological viewpoint – more valid allergen measurement [46]. These studies have probably been successful because wheat allergen levels in flour dust correlate reasonably with dust levels [26, 47]. For most other HMW allergens, however, there often is no such direct correlation between airborne dust and allergen levels, because dust and allergen levels do not always share the same sources and determinants of exposure. For these allergens, exposure-response relationships could be explored only after immunoassays became available for sensitive and specific measurement of these allergens [3]. Overviews of available exposure-response studies [17, 48, 49] show that several allergens, e.g., rat urinary proteins and fungal α-amylase, appear to be potent allergens, and are associated with increased sensitization rates at low exposure levels in the nanogram or even picogram per cubic meter range for as little as a few hours per week [26, 49]. Allergens like wheat proteins seem less potent and sensitization risk increases in the low microgram per cubic meter range [50]. A longitudinal exposure-response study in bakers has confirmed the results of the cross-sectional studies on fungal α-amylase and wheat allergen exposure [51]. Clear-cut exposure-response relationships in humans have as yet not been observed for latex proteins, since few epidemiological studies on latex sensitization have yet been conducted in which exposure was assessed with the use of latex-specific immunoassays.

Effect modification of exposure-response relationships

In most of the above-mentioned studies, modification of the exposure-response relationship by atopy has been considered. The most direct and usually optimal approach to assess effect modification by atopy is a stratified analysis, in which intercept and slope of the exposure-response relations are determined separately for atopic and non-atopic subpopulations ('strata'). In most of the studies following that approach, the slope of the exposure-response relationship is generally steeper for atopics compared to non-atopics.

One should realize that the association between exposure and sensitization is biologically specific in the sense that specific antibodies are formed against the allergen. This implies that there is only one determinant of sensitization in a strict epidemiological sense, because a confounder is required to be a determinant of the endpoint under study. Confounding cannot occur because there is only one determinant, and a confounder is by definition another determinant.

Further along the causal chain, other endpoints can also be studied. Several studies have explored the relationship between symptoms and exposure. This requires a specific strategy because the respiratory or work-related symptoms may not exclusively be caused by exposure to one specific allergen, but may also be associated

with other factors in the work environment, or to non-occupational factors like smoking, exposures at home, or outdoor exposures. Thus, confounding by several other causes may occur here. If one wants to explore the association between exposure and symptoms, one needs to further stratify for specific sensitization because this is expected to be a strong modifier of risk for symptoms elicited by the specific allergen exposure. This is clearly illustrated by the example below (Tab. 1).

Table 1. Exposure-response relationship for work-related respiratory symptoms and wheat allergen exposure stratified by specific IgE sensitization to wheat (from [50]).

	Work-related symptoms	
	n/N	**%**
Sensitization to wheat		
Low exposed	1/7	14.3
Intermediated exposed	4/10	40.0
High exposed	10/19	52.6
Not sensitized to wheat		
Low exposed	17/110	15.5
Intermediate exposed	21/97	21.6
High exposed	25/103	24.3

IgE-sensitized workers had a considerably higher risk of developing symptoms at intermediate and high exposure levels than non-sensitized workers, and the exposure-response relationship in this subgroup is much steeper in the wheat-sensitized subgroup. Interestingly, additional analyses suggested that among non-wheat-sensitized workers, atopics (workers with IgE to common allergens like house dust mites and grass pollen, but not to occupational allergens) also had a somewhat higher risk than non-atopics. Atopic individuals are known to respond more often and possibly at lower exposure levels to any irritant or allergenic stimuli. The underlying mechanism is not associated with specific sensitization to a common allergen, but more likely an indirect association with bronchial hyperresponsiveness or some irritant mechanism. Similar observations are available for other allergens and from longitudinal studies [52, 53]. Some studies have shown findings indicating that sensitization does not always precede symptoms in people exposed to known allergens [54]. Mechanisms than other sensitization probably explain the occurrence of their symptoms. Thus, several exposure-response relationships exist with different underlying biological mechanisms. If one does not distinguish these different modifiers in the analysis, exposure-response relationships become diluted or may

have an unexpected shape because the different associations are superimposed. This may be one of the reasons why several studies in the past have failed to find clear exposure-symptoms relationships because of the complexities and pitfalls. Several other studies explored relationships between exposure and symptoms in a straightforward way without finding a clear relationship. Exposure-symptom relationships can only be studied in a meaningful way when the analysis is stratified for modifying variables along the causal pathway, like sensitization and potentially atopy in the case of HMW sensitizers.

The most evaluated exposure-response relationships in allergic respiratory disease are exposure-sensitization and exposure-symptom relationships in cross-sectional and some longitudinal studies. However, examples in which, for instance, time to sensitization has been evaluated also exist [55]; these have shown that the time to development of disease is also dependent on exposure intensity.

Special issues in exposure-response modeling

Some studies specifically explored the existence of exposure thresholds using smoothing techniques [56–58]. There were no indications of an exposure threshold or exposure level below which the sensitization risk was not increased. Others have found levels below which sensitization did not seem to occur [40]. However, the difference between no or a few cases in the lowest exposed category and thus between observing an exposure threshold or not seems small. The power of a study, the size of the control group, the background prevalence of sensitization of the allergen, and the way sensitization has been measured may be the major driving factors for the estimated sensitization rate at the lowest exposure levels. The difference between studies that do not find exposure thresholds and those that do are to some extent relative and artificial, while the consequences for prevention and exposure standard-setting practices are obvious [59]. For some sensitizers health-based occupational exposure limits (OELs) have been defined that specify the level of exposure to an airborne substance, a threshold level below which it may reasonably be expected that there is no risk of adverse health effects. When such a threshold does not exist, an alternative approach would be to accept a low exposure, which carries a small but predefined risk in developing allergic sensitization.

For several allergens, a flattening of the exposure-response relationship at higher exposure levels has been reported [57, 58]. Some have argued that flattening off of the exposure-response relationship may be associated with specific IgG and IgG4 responses at high exposure [60, 61]. Others observed the same phenomenon in independent studies [62, 63]. However, this phenomenon was not observed in a longitudinal study looking at IgG antibody response at baseline and incidence of specific sensitization [64]. In one study it was found that the exposure-response relationship for wheat exposure with specific sensitization differed from industry to

industry, possibly reflecting a differential selection bias (healthy worker effect) by industry [57]. More studies are needed to understand the underlying explanations for flattening of exposure-response relationships at high exposure levels.

Exposure-exposure interactions

Some environments with high levels of HMW allergens also appear to be associated with a strongly decreased risk of common atopy. Interactions seem to exist between exposure to microbial agents like endotoxins and sensitization. Studies among adults, some with occupational exposure data for microbial agents such as endotoxin, have shown inverse relationships between endotoxin exposure and atopic asthma [65], allergic sensitization [66], and hay fever [67, 68]. This suggests complex and interacting associations between endotoxin and allergen exposures in adult life. Similar complex interactions seem to exist with other exposures such as disinfectants [12]. Evaluation of interactions as described above will become more often the norm and not the exception. This will ask for studies with refined exposure-assessment strategies and sufficient power to be able to distinguish the effect of the different exposure variables on different phenotypes. An examples of this development will be a new generation of gene-environment studies.

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