

Effect of Voorhorst's work on the current understanding of the role of house dust mites in allergic diseases

Voorhorst's article of 1967 provided conclusive support that a little-recognized species of house-dwelling mite, *Dermatophagoides pteronyssinus*, was the origin of the elusive and ubiquitous house dust allergen. The article was followed 2 years later by a book¹ that comprehensively presented this case backed by extensive data and 168 references and that became a manual for those who continued this line of research.

The article forms the critical initial point in the progressive growth of publications about mites and their role in allergic diseases (Fig 1). Within this, 2 phases can be identified. Between 1967 and 1984, interest grew slowly to about 40 publications per year. By this time, the global distribution and biology was well established, the global ubiquity of mite allergy had been confirmed, several major mite allergens had been purified, specific immunoassays for these allergens were in use, and a major clinical role for mite allergen exposure in driving the underlying airways hyperresponsiveness had been substantiated. From 1984 until the end of the millennia, there was a marked increase in the rate of publications. This was consistent with the enthusiasm that greeted the First International Workshops in Bad Kreuznach in 1987² and the Second Workshop in Minster Lovell in 1990.³ However, by the time of the Third Workshop, in 1995,⁴ the field had broadened to include other indoor allergens, and although the science was advancing, so was the complexity. Aspects such as the support for threshold levels for exposure and symptoms were diminishing, and along with it, the simplistic overview that "mite allergens were the cause of asthma" diminished as well. A fourth workshop would have been due by 2000 but was never held. By then, the field had broadened further, and the pendulum of etiologic interests had swung to factors that were actively protective against asthma rather than factors that induced the disease. Indeed, there is a recent view that allergy and allergen exposure accounts for only 40% of the population-attributable risk of asthma, with mites accounting for only 4%.⁵ However, by now mites are firmly established in the public mind. A search on "dust mite*" in Alta Vista finds over 66,000 hits. A final note of respectability has occurred in the last 2 years with the record sales of globally marketed cleaning products that offer mite and cat allergen avoidance. Mite allergens are mainstream big business. This is in contrast to the emerging research interests in the potential benefits of microbial exposure and high-dose allergen tolerance. Colloff et al⁶ and Arlian and Platts-Mills⁷ provide useful state-of-the-art reviews of mite biology.

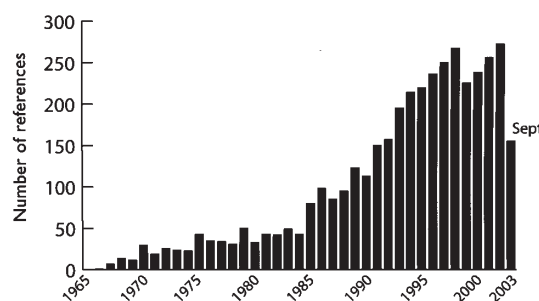


FIG 1. Bar graph of the number of publications for each year from 1960 through 2003 found by using the search terms "mite or mites" and "allergen or allergy" in Medline. Note that 2003 data are only until September.

The work of Voorhorst and colleagues brought together threads of research in both house dust allergy and mites conducted since the 1920s in Holland. Their success was grounded on 2 factors: first, an appreciation of the extrinsic components of allergic diseases, which linked allergy, rhinitis, asthma, and eczema with the patient's environment, and second, the use of quantitative skin testing with serial dilutions of extracts to determine their allergen content. Both of these have reflections in the renewed interest in the nose-lung connection and in the increasing insistence on rigorous outcomes-driven study design and statistical analysis of data.

The article focuses on 6 aspects: (1) the ubiquity of allergy to house dust mites; (2) the existence of a single major house dust allergen; (3) the variability in this allergen between different houses; (4) the fact that this allergen is found in a variety of sources within a house; (5) the concordance of the late summer seasonality of patients with dust allergy and the allergen content of dust; and (6) the adverse effect of damp and poorly constructed houses on a subject's asthma. These will be discussed in the context of recent developments before considering additional progress in the field.

It can be speculated that there is more specific IgE directed toward mite allergens than to any other single allergen source in the world. This is based on the combination of the global prevalence of mite allergy and the high proportion of the total IgE directed toward these allergens. Studies in many countries have demonstrated that the prevalence of mite allergy in temperate climates reaches 60% to 80% of children with asthma and up to 40% in the general population, most of whom have no disease. It has further been shown that 6% to 21% of total serum IgE is specific for mite allergens and that 50% to 80% of this is directed at just 2 allergens, Der p 1 and Der p 2. Although human IgE response to mite allergens is dominated by responses to Group 1, 2, and 3 allergens (probably in that order), curiously, animals such

as dogs and cats have much stronger responses to Group 15 mite allergens. However, we still do not know why some allergens and not other inhaled mite proteins generate a specific IgE response. In addition to IgE, most persons possess detectable T-cell responses to mite allergens, with greater responses associated with the allergic phenotype T_H2 in allergic subjects. Other nonallergenic proteins found in extracts include an abundant, soluble, 40-kd cuticle protein and mite ferritin, which do not induce an IgE response, despite ferritin being a potent stimulator of T_H1 and T_H2 T cells.

Consistent with the above, it can also be speculated that mite allergens are probably the single most ubiquitous allergen in the world. The 1967 article lists 14 countries in which mites were found. However, it would be exceptional to find a country in which no house dust mites were reported. Even in desert or cold regions, in which mites are rare, small pockets of mites will occasionally survive in a few beds, or the allergen is carried there from other sources. Colloff⁸ has accumulated the most comprehensive database of occurrences of the 11 most frequently occurring dust mite species, currently listing 75 countries and more than 700 localities in which dust mites have been recorded in homes. These range in latitude from Sr-Varanger in Norway to Punta Arenas in Chile and up 3670 m in altitude. For localities for which quantitative data exist (currently $n = 140$), the overall mean occurrence of mites is 88.2% of samples. For localities in which allergen Der p 1 levels have been quantified (currently $n = 85$), the mean occurrence is 86.8%.

By 1969, Voorhorst considered that the same "house dust allergen" was contributed by the mite species *D pteronyssinus*, *Dermatophagoides farinae*, and *Euroglyphus maynei* and not by a range of other storage mites. It was unclear to contemporaries whether the allergen was largely polysaccharide or glycopeptide, and it was commented that "the pure allergen occurs in such limited quantities that its chemical composition is completely camouflaged." The most recent data on the occurrence and interrelationship between the different mite allergens is reviewed by Thomas et al.⁹ They catalogue 19 groups of allergens associated with domestic mites, 16 of which are found in either *D pteronyssinus* or *D farinae*. The relationship between the naturally occurring allergens and their recombinant analogues is still evolving, and in some cases, more is known about the recombinant than the native allergens (eg, Groups 5 and 8). The earlier analytic approach of using protein blotting to identify naturally occurring allergens showed approximately 30 IgE-binding bands. However, interpretation is confounded by the lability of conformational epitopes, the effects of natural degradation, the variations between sources, and the

occurrence of some allergens with multiple molecular weights and of different allergens with the same molecular weight.

Voorhorst and colleagues explored the complexity of domestic environmental agents that were responsible for asthma exacerbations. This initially involved monitoring the correlation between season and the fluctuations in asthma exacerbations and the allergen content of dust. They also showed, through skin testing with cultured fungi and bacteria, that these allergens were not of primary importance, nor was such allergy common. Interestingly, they noted some extracts of *Bacillus* species and yeasts elicited immediate skin responses, but the responsiveness could not be transferred with serum in the PK test. This foreshadows current interest in the inflammatory roles of zymogens, glucans, and endotoxins and in nonallergic rhinitis and asthma symptoms that are specific to locations. Although recent meta-analyses have shown significant asthma risk associated with fungi, other researchers have struggled to clearly establish a dose-response relationship between asthma symptoms and exposure, and the most convincing data remain from studies in arid climates, in which mite allergens are of little importance.

Voorhorst was also intrigued by the potential contribution of human dander. He was convinced this was an important allergen and was able to show that such activity also did not correlate to mite content of dust and that it was both distinct and less. Although its importance continues to remain elusive, there is clear data indicating IgE binding to recombinant human epidermal proteins. Additional allergens from fungal or staphylococcal contamination of skin are also plausible sources of such allergic responsiveness.

Voorhorst has been credited with originally proposing an association between mites per gram of dust and the risk of allergic disease. Such a pattern was proposed in numerous subsequent studies (eg, Korsgaard) and was translated in equivalent micrograms of allergen per gram of dust, which was adopted as a threshold value by the First International Workshop. However, such propositions were not directly made, and his repeated observations were of more asthma associated with houses in damp areas of the city of Leiden and the seasonal association of both asthma and rhinitis symptoms and increased mite numbers (ie, change in exposure rather than thresholds). In some of the houses, the peak mite numbers involved were modest.

Once the role of mites became apparent, then the link between mite physiology and both the seasonality of the allergens and the risk associated with damp houses could be understood. Although their experiments outlined the importance of temperature and humidity on the growth of mite cultures, the com-

plexity of developing quantitative ecologic models of why mite allergen levels differ between houses in the same town or between rooms in a house remains. Factors such as housing type, building "tightness," storey height, capacity of materials to retain moisture, and possession of air conditioning all affect microclimate and thus mite activity. Other factors, such as protein and lipid content of dust or the effects of pheromones and other arthropods and fungi present, are all underexplored. On the basis of appreciation of the critical role of water balance in mite ecology, a number of low-allergen houses were constructed in Denmark by using mechanical ventilation with heat exchange, which provided clinical benefit to subjects with asthma. There have been numerous attempts at successful domestic allergen avoidance both before and after Voorhorst, and despite wide advocacy, compliance has been poor. Given sufficient allergen reduction, many patients do show a broad range of clinical benefits, but it is also clear that single interventions do not usually provide sufficient reductions to provide benefit. This area requires further development. Medications can only provide short-term control of symptoms; the long-term goals of research should include primary or secondary disease prevention and modulation of its immunology.

The article also describes recovering allergen from glass beads in which mites had been maintained without food and that dust contained 20 to 30 times more mite allergen than could be accounted for by mite numbers, concluding that the allergen was secreted or excreted. This is consistent with the known allergen content of mites and their feces, IgE immunostaining of thin sections locating allergens in the mite gut, and the enzymic function of allergens Groups 1, 3, 4, 6, and 9. These enzymes are hypothesized to contribute to the allergenicity by degrading epithelial tight junctions in the airways, thus improving permeability and directly influencing immune function through cleavage of CD23 and CD25.

Voorhorst and colleagues could never have imagined the radical effect that developments in genomics and proteomics would have on our understanding of mites at a molecular level.^{9,10} These developments include the creation and analysis of cDNA libraries, studies of genomic DNA and expressed sequence tags, and application of matrix-assisted laser desorption-ion ionization and tandem mass spectroscopy to the highly resolved proteins on gels. Mites probably express approximately 10^5 genes, of which approximately 3% code for proteins. Bioinformatic analysis indicates that about 30% of mite proteins are novel, with no recognized function, whereas others show homology to common structural, biosynthetic, signaling, cell surface, or metabolic proteins, such as actins, cuticle-like proteins, esterases, kinases, ferritin, and others. About 5%

relate to allergens. Such analysis has contributed 2 major insights. First, at a molecular level, there is an extremely high level of sequence polymorphism for the allergens, including for Groups 1, 2, 3, and 5 thus far. Indeed, 3 of the reported 7 epitopes of Der p 1 possess polymorphic residues, and the polymorphisms of Der f 2 include regions previously assigned to T-cell epitopes. Second, mite allergens possess numerous structural homologs, not only with other mites also but with some insects, pollens, fruit, and fungi, which might give rise to panallergens right across taxonomic kingdoms.

Ultimately, such analysis will provide new understanding of mite biology, indicating ways of controlling mite populations, and provide novel approaches to therapy through analysis of the immunoresponse. Already prototype diagnostics are operating at this molecular level, at which hundreds of synthetic molecular fragments are microarrayed on chips to allow the analysis of IgE specificity for this molecular diversity. Many pathways are being explored for modulation of IgE responses by either anergising specific T cells or downregulating the IgE response. These include recombinant allergens modified to minimize IgE responses, the use of peptide fragments of allergenic molecules or altered T-cell ligands, DNA vaccines that are delivered through plasmids, and allergens conjugated to T_H1-directing CpG (bacterial) DNA.

The 1967 article and subsequent book were seminal in providing background, data, and techniques to allow mite allergy to be explored elsewhere in the world. Yet the book is difficult to locate. A search of the OCLC database found only 24 copies held by approximately 8000 US university hospital and medical libraries; 1 copy was available in Australia, and none could be located in Canadian (CISTI), British Library, or German (SUBITO) databases. The largest aggregation of Web sites for secondhand books (www.abe.com) contained only a single copy for sale.

By all accounts, Voorhorst was a determined character and not a man who suffered fools. His conviction of the importance of the domestic environment in asthma etiology must have been strong to persist in recruiting hundreds of patients in such studies, in collecting thousands of dust samples plus microbial cultures, in recruiting biologists, and in conducting thousands of serial skin tests and pushing forward his ideas. Even though today his name is not as well known as it might be, personally, he would have to acknowledge with pride and satisfaction where the path that he started has led.

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