
Standardized Extracts, Dust Mite, and Other Arthropods (Inhalants)

Annette W. Ford and Thomas A. E. Platts-Mills

The role of pollens, mammalian danders, and house dust in the provocation of asthma and allergic rhinitis has long been recognized. The recognition of arthropods, such as mites and insects, as important sources of inhalant allergens is more recent, and it was only in 1964 that house dust mites of the genus *Dermatophagoides* were shown to be the most common source of house dust allergen.

This chapter discusses the progress of knowledge of allergy to mites including consideration of house dust allergy, the identification of allergens, and the development of an International Standard (IS) for *D pteronyssinus* extract. In addition, the availability, safety, and efficacy of diagnostic and therapeutic procedures used in house dust allergy are discussed with a summary of clinical trials of mite extracts to date.

History and Epidemiology of Arthropod Allergies

Over 1 million species of arthropods exist, of which the vast majority are insects, arachnids (approximately 50,000 species), and acari (ticks and mites, some 40,000 species).¹ Characterization of the acari are particularly incomplete and it is thought that only a small proportion of species that exist have been classified. Only a small proportion of arthropod species are known to produce inhalant allergens. The first allergic disorder attributed to mites was an outbreak of "epidemic asthma" in grain workers.² The cause was the predatory mite, *Pyemotes (Pediculoides) ventricosus*, which feeds on the larvae of insects that infest grain and straw.

Table 1 shows some of the mites and insects commonly encountered in allergy and indicates their taxonomic relationships. The most frequently implicated arthropods in allergy are the Pyroglyphid mites of the genus *Dermatophagoides*. These mites are so commonly found in house dusts that they have become known as "house dust mites." Most are of the species *D pteronyssinus* (Fig. 1), *D farinae*, and *D microceras*. The species *D pteronyssinus*, identified in house dust by Fain,⁶ was shown to be very common by Voorhorst et al⁷ who demonstrated a close correlation between the number of mites and

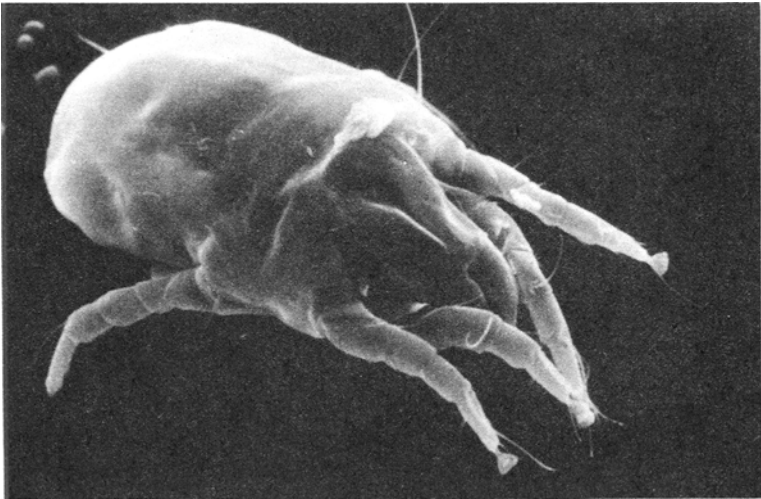
From the National Institute for Biological Standards and Control, Holly Hill, London NW3, 6RB, England; and the University of Virginia, School of Medicine, Charlottesville, VA 22905.

Table 1. Relationships Between Athropoda of Allergenic Potential

Class	Order	Family	Genera & species	Common name
Arachnida	Astigmata	Pyroglyphidae	<i>Euroglyphygus maynei</i> , <i>Dermatophagoides</i> <i>pteronyssinus</i> , <i>D farinae</i> , <i>D microceras</i>	Dust mites
		Acaridae	<i>Acarus siro</i> , <i>Tyrophagus</i> <i>putrescentiae</i> , <i>T longior</i>	Storage mites
		Glycyphagidae	<i>Glycyphagus domesticus</i> , <i>G destructor</i>	
	Prostigmata	Cheyletidae	<i>Cheyletus troussarti</i>	Predatory mites
		Tarsonemidae	<i>Tarsonemus</i> spp	Damp dust mites
		Pyemotidae	<i>Pyemotes ventricosus</i>	Predatory mites
Insecta	Orthoptera	Acridoidae	<i>Locusta migratoria</i>	Locust
	Dictyoptera	Blattidae	<i>Periplaneta americana</i> , <i>Leucophaea maderae</i> , <i>Blatta orientalis</i> , <i>Blattella germanica</i>	Cockroaches
	Coleoptera	Curculionidae	<i>Tribolium confusum</i> <i>Sitophilus granarius</i>	Flour beetle Grain weevil
	Diptera	Chironomidae	<i>Chironomus riparius</i> (<i>C thummi</i>) <i>Cladotanytarsus lewisi</i>	Midges, bloodworm "Green nimitti"
		Muscidae	<i>Musca domestica</i>	House fly
		Calliphoridae	<i>Calliphora</i> spp	Bluebottle
			<i>Cochlionyia hominivorax</i>	Screwworm
	Hymenoptera	Apidae	<i>Apis melliphora</i>	Honey bee
	Lepidoptera	Bombycidae	<i>Bombyx mori</i>	Silkworm moth

From Blythe,³ Wharton,⁴ and Bellas.⁵

Figure 1. Scanning EM of *D pteronyssinus* with fecal particles.



the allergenicity of house dust. Since then mites have been found in the house dust of every country in the world where their presence was sought.⁸⁻¹³ Furthermore, in a tropical area where mites were previously rare, an increase both in the number of *D pteronyssinus* and in cases of asthma occurred when blankets were introduced.¹⁴

In individual numbers the Pyroglyphidae usually exceed the numbers of all other arthropod groups put together, forming 70 to 92% of the mites from floor and carpet dust and 90 to 97% of those from domestic mattress dust in the United Kingdom.³ Japanese workers found 36 different mite species in house dust.⁹

D pteronyssinus is the predominant species in houses in the United Kingdom and in Northern Europe, whereas *D farinae* tends to replace it in areas that have larger seasonal changes in relative humidity. The latter is common in the United States¹⁵ but is rarely encountered in the United Kingdom. As many as 3000 mites/100 mg have been found in dust from mattresses,³ whereas Korsgaard¹⁶ has suggested that greater than 100 mites/gram of dust should be regarded as a risk factor for the development of mite allergic asthma.

Dermatophagoides mites, as their name suggests, live on human skin scales, which form a major part of house dust—an adult human sheds up to 5 g of skin scales per week.¹⁷ Other groups of mites including *Acarus siro*, *Tyrophagus putrescentiae*, *Glycyphagus domesticus*, and *G destructor*, which are often referred to as storage mites, are mainly pests of stored grain but have been held responsible for “barn allergy” in farm workers.¹⁸

In 1967 Voorhorst et al⁷ showed that *D pteronyssinus* fulfilled all the necessary requirements to be the major source of allergen in house dust; ie, ubiquitous distribution, specificity, seasonal variation in allergen content, and present in high enough concentrations. Although other biologic materials in house dust such as mammalian dander, pollen, and cockroach allergens also contribute, *Dermatophagoides* mites are now generally acknowledged to be the most common allergenic source. A correlation between mite numbers and allergic disease has been described in the Netherlands for *D pteronyssinus*¹⁰ and in Canada for *D farinae*.¹⁹

Although insects such as cockroaches, beetles, and flies contribute to house dust, they are better recognized as causes of occupational allergy²⁰: for example, grain weevil (*Sitophilus*) allergy among grain handlers,²¹ allergy to locust debris among research workers in the United Kingdom, United States, and Europe,²² and screwworm fly allergy among air crew working on a pest control program.²³ Beekeepers, in addition to risking anaphylaxis as a result of a sting, often develop inhalant allergy to beehive dust.²⁴ Cases of occupational or recreational allergy have occurred in the persons who breed fly maggots and in those who use the maggots for bait in fishing.²⁵ Similarly, the use of bloodworms (the larvae of chironomid midges) as fish food in aquaria has resulted in allergies amongst those preparing and using them.^{26,27}

Allergy to naturally occurring midges is common in Minnesota and other areas with large areas of shallow water where midges can breed. Interestingly the most striking disease of this kind, ie, Green Nimitti disease, appears to have developed because man-made changes to the Nile in Sudan created large breeding areas for the midges.^{25,28}

The only insect that is well established as cause of allergy in domestic situations is the cockroach. Reports are common in the United States²⁹ and, in some urban areas, cockroaches may well be as important as dust mites.³⁰

Preparation of Extracts

The natural habitat of *D pteronyssinus* is house dust that usually contains ample quantities of human skin scales. For house dust extracts, as opposed to dust mite extracts, commercial manufacturers use house dust collected with vacuum cleaners and have their own requirements as to the situations in which this dust should be collected. Ideally, these houses should not have pets, nor should the dust be collected at the time of year when grasses are pollinating. In addition, house dust should be collected from a variety of areas and types of housing. In this respect clinical experience suggests that commercial house dust extracts in the United States do not contain significant quantities of cockroach allergen. However, most commercial house dust extracts contain measurable cat allergen as well as dust mite allergen.

For extracts of *D pteronyssinus* mite, live mites, originally obtained from bedding, are grown under controlled conditions of temperature and humidity. A number of authors have claimed that it is easier to culture *D farinae* than *D pteronyssinus* because the former will grow on stored food. For this reason in the United Kingdom until the early 1970s only *D farinae* mite extracts were available, although *D pteronyssinus* was the predominant species. It is now possible to grow either mite on human skin scales or on heat-denatured media, and in most countries (except the United States) both types of extracts are available. For skin testing, because of close antigenic similarity between the 2 species, it appeared possible to substitute *D farinae* for *D pteronyssinus*.³¹ Although it is now clear that there are many species-specific epitopes on mite allergens, it still appears that overall there is extensive cross-reactivity and that most but not all patients are equally allergic (or nonallergic) to both mites.

Culture Media and Conditions

Spijksma³² found that human skin scales mixed with an equal weight of dried powdered yeast, *Saccharomyces cerevisiae*, was the best medium for growth of *D pteronyssinus*. He examined several media containing epidermal scales from cat, dog, horse, cow, rabbit, Guinea pig, sheep, goat, mink, and duck. All sources (except sheep) sustained growth and provided extracts of comparable allergenicity, however, most mammalian danders, other than human, contributed allergens of their own to the mite extract. Indeed in 1981 Aas³³ reported the presence of horse allergens in commercial extracts. Other culture media for *D pteronyssinus* include powdered milk, dog meal, wheat germ flakes each plus 50% (weight/weight) (wt/wt) dried yeast, *Daphnia*, fishmeal plus dried yeast at 17% (wt/wt), wholemeal flour and powdered yeast, and horse dander.³ Today a number of different culture media for growth of *D pteronyssinus* mites are in use, although the media used by many of the manufacturing companies remain commercial secrets.

Of these possible media, the most simple one is that of human skin scales,

which is also unlikely to provide an end product contaminated with irrelevant allergens. However, many commercial extracts today are grown on denatured or partially synthetic media and do not contain detectable allergens derived from the culture medium.³⁴ In assessing the relative merits of different culture media, it is worth considering whether or not human proteins are desirable in an extract, and also whether or not the culture medium itself may have enzymatic activity (R Thorpe, personal communication). At present there is no published formula for an ideal culture medium.

Culture of *D pteronyssinus* mites can be performed at temperatures of 20 to 25°C and at relative humidities of 70 to 80%.¹⁰ The optimal conditions for *D farinae* are slightly hotter and less humid. *D pteronyssinus* is prone to desiccation at lower relative humidities and cultures are susceptible to the overgrowth of moulds at relative humidities of 85% or greater.

Extraction of Material

Mites can be separated from the medium in which they are cultured by flotation or live mites can be encouraged to migrate from the culture medium by creation of a temperature, humidity, or light differential. Alternatively, the whole outgrown culture, which will include both live and dead mites, mite parts, feces, larval forms, nymphs, and eggs, and any unconsumed growth medium, can be extracted. The live mites can be killed before extraction by dry heat, an atmosphere of acetone, or by freeze-drying.³⁵ Extraction is invariably in an aqueous buffered solution, such as Coca's solution (0.25% NaHCO₃ + 0.5% NaCl), ammonium bicarbonate, or a glycerol saline solution. Phenol is often included at 0.4% as a preservative if the extract is not to be freeze-dried. Extraction time used commercially is normally 24 hours. However, recently it has been demonstrated that maximal liberation of IgE-binding components of *D pteronyssinus* takes place within 1 hour of extraction³⁶ and that the bulk of the major allergen, antigen P₁ itself, is released within 3 minutes from mite cultures.³⁷

Characterization and Cross-Reactivity of Arthropod Allergens

The characterization of allergenic extracts uses a variety of methods well described in the literature, for example, the radioallergosorbent test (RAST),³⁸ RAST inhibition,³⁹ crossed-immunoelectrophoresis (CIE), and crossed-radioimmuno-electrophoresis (CRIE),⁴⁰ radioimmunoassay (RIA),⁴¹ and histamine release (HR).⁴² Many of these were used in the collaborative study for the IS for *D pteronyssinus* mite, where the intention was to encourage the use of as many different, valid techniques as possible. The purpose of characterizing extracts is usually to quantify the antigens present, to identify the components most commonly responsible for causing allergy and, where possible, to purify the important allergens. It should then be possible to examine the main sources of those allergens and to ensure that individual allergens are present in the extracts used for diagnosis and immunotherapy.

Extracts of whole cultures of *D pteronyssinus* have been shown by CIE to contain as many as 49 Coomassie Blue staining, immunoreactive proteins

with an additional 2 antigens revealed by CRIE making a total of 51 separate antigens. At least 9 of these bind IgE antibody and are thus considered as allergens.⁴³ Using immunoblotting techniques up to 20 antigens appear to bind IgE.⁴⁴ However, it is very difficult to confirm: 1) that each of the protein bands are distinct allergens, and 2) that the binding represents specific IgE antibody.

Antigen P₁, present predominantly in mite feces, was isolated and characterized in 1980 by Chapman and Platts-Mills.⁴¹ Isolation was by Pevikon block electrophoresis and it was shown to be a glycoprotein of molecular weight of 24,000 daltons and with isoelectric points (pIs) of 4.7 to 5.4 and 6.6 to 7.1. The same allergen has been isolated independently as Ag 42 by Lind et al^{43,45,46} and as Dpt 12 by Stewart and Turner.⁴⁷

The identity of these allergens was clearly demonstrated in the collaborative study for an IS⁴⁸ by the close agreement of results obtained by different workers using different assay methods. Further evidence of the identity of antigen P₁ and Ag 42 is presented by Lind et al⁴³ and in Figure 2. Another mite protein that appears to be a major allergen, now partially isolated, is antigen DpX⁴⁹ (called Antigen 17 in this reference).⁴⁵ This protein with a molecular weight of 18,000 to 20,000 daltons has been shown to be quite distinct from antigen P₁, to be present in the feces and mite bodies and, like P₁, to bind to IgE present in the serum of the majority of mite-allergic subjects using CRIE. Antigens Y and 23 are considered as intermediate allergens (ie, ones not of major importance for the majority of patients). However, they may be of importance in standardization because they are found in relatively higher concentrations in pure mite body extracts than in those made from whole culture or mite feces.^{43,48}

To avoid confusion where different workers have isolated and separately named important allergens, a new system of nomenclature has been proposed that uses the first 3 letters of the generic name, the initial letter of the specific name, and the Roman numerals I, II, and so on, depending on the relative importance or, where this is equal, on the order of isolation.⁵⁰ Thus antigen

Figure 2. Identity of antigen P₁ and Ag 42 by tandem CIE. 4µg of antigen P₁ (left) and Ag 42 (right) were placed in wells in agarose and electrophoresed for 30 minutes at 10 V/cm. Electrophoresis at right angles to the first dimension was performed into agarose-containing rabbit antisera to *D pteronyssinus* extract for 16 hours at 2 V/cm. The gel was stained with Coomassie Brilliant Blue R.

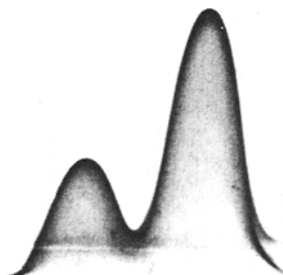


Table 2. Important Allergens Isolated and/or Characterized From Arthropods: *Dermatophagoides*

New name	Name given by author	Characterization or separation method	Nature	MW	pI	Reference
<i>D pteronyssinus</i> Der pl	F ₁ P ₁	Gel filtration & electrophoresis	glycoprotein	15,000–25,000 24,000	ND 4.7–5.4 & 6.6–7.1	51 41
	P ₁	Gel filtration, electrophoresis & preparative IEF				
	AgIV	CIE/CRIE				
	Ag20	CIE/CRIE				
	Dp42	Hydrophobic interaction chromatography & copper chelate chromatography				
	Dpt12	Gel filtration				
	Dpt12	Chromatofocusing				
	Dp7	CIE/CRIE				
	AgII	CIE/CRIE				
	Ag17	CIE/CRIE				
Der pII	DpX	Gel filtration, (NH ₄) ₂ SO ₄ ppt	ND	18,000–20,000	ND ND 5–7	52 49 45
		hydrophobic interaction chromatography				
	DpY	CRIE				
	AgI	CIE/CRIE				
	Dp23	CIE/CRIE				
<i>D farinae</i> Der fl	Dpt4	Gel filtration	glycoprotein	274,000 ND	4.5 ND	47 56
	Dpt4	Con A affinity chromatography				
	Dpt 4	Preparative ultracentrifugation, PAGE				
	AgI	CIE, ammonium sulphate ppt DEAE chromatography				
	AgF ₁	Monoclonal antibody				
<i>D microceras</i> Der ml	Df6	Polyclonal antibody	ND	ND	ND	59, 60 61
	Dm6	Polyclonal antibody				

ND = not determined; MW = molecular weight
 CIE = crossed-immunoelectrophoresis; CRIE = crossed-radioimmuno-electrophoresis; IEF = isoelectric focusing; ppt = precipitation; PAGE = polyacrylamide gel electrophoresis; Con A = concanavalin A; and pI = isoelectric point.

P₁ (= Ag 42 = Dpt 12) is now to be known as Der pI and AgX as Der pII (Table 2).

Major allergens for *D farinae* and *D microceras* (Der fI and Der mI, respectively) have been described for *D farinae* (Ag 11 of Dandeu et al)⁵⁸⁻⁶⁰ and for *D farinae* (Df 6) and *D microceras* (Dm 6).^{53,62} Partial identity has been shown to exist between Der pI and Der fI by crossed-line immunoelectrophoresis (CLIE), crossed-inhibition in RAST, and amino acid composition.^{53,60,62} Recently detailed analyses of the allergenic molecules have been made with monoclonal antibodies or affinity purified antibodies, and it is clear that epitopes common to the major allergens of all 3 species exist, as well as species-specific determinants and determinants shared with only two of the 3 species.^{59,62-64} Use of the species-specific monoclonal antibodies or affinity purified antibodies against these major allergens is proving to be a better way of quantitating the individual species in house dust than microscopic examination and identification of the mites.^{61,64}

The storage mites are taxonomically distinct from the Pyroglyphid mites (Table 1). The species *Acarus siro*, *Tyrophagus longior*, *T putrescentiae*, and *Glycyphagus destructor* show multiple precipitin arcs when studied by homologous CIE using rabbit antisera raised individually against the extracts,^{65,66} however, when extracts of these mites are electrophoresed into agarose-containing rabbit antisera raised against *D pteronyssinus* or *D farinae*, only 1 to 3 precipitin arcs are seen.⁶⁶ Similarly, 3 or 4 antigens are shared by, or have partial identity within, 3 storage mite species.^{66,67} Patients exhibiting positive skin tests to both the pyroglyphid and storage mites appear to do so because of multiple sensitization to the different species rather than because of cross-reactive antibodies.

Compared with the mites very few of the inhalant insect allergens have been characterized and even fewer major allergens have been purified. However, the location of major allergenic activity of the chironomid midges (*Chironomus riparius* = *Chironomus thummi thummi*) has been investigated and found to be in the hemoglobins, the erythrocrurins, 11 of which have been isolated.²⁶ A number of these erythrocrurins (CTT IV, CTT VI, and CTT VIII) have been cleaved and the fragments sequenced and found to be antigenic. Immunologic cross-reactivity was found to exist between *Cladotanytarsus lewisi* and 4 species of *Chironomus*,²⁷ and this finding has been further confirmed between the 2 genera by CIE, RAST inhibition, and positive skin prick tests.⁶⁸

Safety and Efficacy of Arthropod Extracts for Diagnosis and Therapy

Mite Extracts

Diagnosis. A number of companies market extracts of house dust mites supplied in 50% glycerol saline for skin prick testing and aqueous extracts for intradermal or bronchial and nasal provocation tests (Bencard, Merck, Miles, Pharmacia in the United Kingdom; Hollister-Stier, in the United States). All are preserved with approximately 0.5% phenol. The concentration or

potency of these are expressed in percentage weight/volume (wt/vol), protein nitrogen units (PNU) or in biologic units (Table 3). As yet, none of the companies are expressing potency in international units (IU) and, until this is the case, it is very difficult to compare the potencies directly. However, when tested in RAST inhibition, the diagnostic extracts for *D pteronyssinus* currently available in the United Kingdom have activities similar in potency to each other and to that of the International Standard.

It is important to realize that positive skin tests to mites may occur in as many as 14% of the general population and many of these individuals have no symptoms.⁷⁰ However, Smith et al³¹ showed that the majority (ie, >75%) of asthmatic children had positive skin tests to *D pteronyssinus* or *D farinae*, and that fewer of them gave positive skin tests to extracts of house dust itself, grass pollens, or those of mammalian dander, food, or moulds. Maunsell et al⁷¹ and Pepys et al⁷² also reported a very high frequency of positive skin tests to mites among patients with asthma who react to house dust. Korsgaard¹⁶ has gone a step further in defining a particular concentration of mites in the house of a mite-allergic patient, which represents a risk factor for the development of asthma.

Skin prick testing, which introduces nanolitre amounts of extract into the epidermis, is quick and safe, but local effects such as itchiness and the occasional late local reactions can occur. Intradermal skin testing carries the same risks with mite extract as with any other potent allergen extracts.

Therapy. When house dust mite is considered to be a major contributory factor in a patient, a number of measures in the house are usually recommended before recourse to hyposensitization therapy. Improvements can be made by the removal of dust-harboring furnishings such as carpets and chairs and of furry toys from childrens' bedrooms by thorough cleaning or even replacement of mattresses or by frequently washing the bedding in hot water. These measures can result in some amelioration of asthma and nocturnal wheezing.^{73,74} Plastic casing for mattresses alone has given reduction in symptoms⁷⁵ as have fungicides that destroy the moulds on which some

Table 3. Details of Mite Allergen Products for Diagnosis Available in the United Kingdom

Company	Product name	Species available	Description of product	Units
Bencard	Bencard skin testing solutions	<i>D farinae</i> <i>D pteronyssinus</i>	Prick tests: in 50% glycerin, 6 % NaCl Intradermal tests in dilute phosphate buffer	wt/vol as percentage
Miles (Dome/Hollister-Stier)	Allergen extract	<i>D pteronyssinus</i>	Prick tests in 50% glycerol	PNU/mL
	Dialyzed allergen extract	<i>D pteronyssinus</i>	Prick tests in 50% glycerol	U/mL
E Merck Ltd	Merck skin testing solutions	<i>D pteronyssinus</i>	Prick tests in 50% glycerin, 0.5% NaCl	PNU/mL

Information taken from ABPI Data Sheet Compendium, 1985,⁶⁹ and labels.

mites feed, or acaricides that kill the mites themselves.⁷⁶ Relief from symptoms has been observed on removal of the patient to areas where mites are rare, such as the hospital.⁷⁷ Some patients obtain dramatic relief on moving to a new house, especially to newer buildings in less damp situations. However, reducing mite allergen in houses is not simple and 2 studies have concluded that standard recommendations to patients do not help, because they fail to reduce mite numbers or allergen levels.^{78,79}

Because of the difficulty in cleaning or changing houses many patients also are treated with hyposensitization therapy. In Europe there are a variety of extracts for hyposensitization available to the practicing physician (Table 4). These include aqueous extracts, aqueous extracts adsorbed onto either aluminium hydroxide, sodium alginate, or tyrosine suspensions, and pyridine extracts that are alum-precipitated. As in the case of skin testing solutions, the potencies are, as yet, expressed in different units depending on the com-

Table 4. Details of Allergen Products for Hyposensitization Available in the United Kingdom

Company	Product name	Description	Dosage form & units	No of doses
Bencard	Alavac-S	Alum-precipitated, aqueous pyridine extract of specially prepared allergens for the individual patient. <i>D pteronyssinus</i> available	3 multidose vials: no. 1, no. 2 & no. 3, containing 10-fold concentrations	8-9
	Migen	Tyrosine-adsorbed, glycerinated extract of <i>D pteronyssinus</i>	6 prefilled syringes containing 0.5 ml of vaccine in doses of 4-400 NU/mL	6
	SDV (specific desensitizing vaccine)	Aqueous desensitizing vaccine individually formulated. <i>D farinae</i> & <i>D pteronyssinus</i> available	3 multidose vials: no. 1, no. 2 & no. 3, containing 8-fold concentrations	12-18
Miles (Dome/Hollister-Stier)	Allpyral allergen extracts	Pyridine-extracted, alum-precipitated extracts specifically prepared for the individual patient. <i>D pteronyssinus</i> & mite fortified house dust available	3 multidose vials: no. 1, no. 2 & no. 3 containing 100, 1000, & 10,000 PNU/mL ^a (<i>D. pteronyssinus</i> only—no. 3 4000 PNU/mL)	9-10
	Conjuvac Mite	Dialyzed, lyophilized extract of <i>D pteronyssinus</i> mite conjugated to alginate & freeze-dried	12 single dose vials of allergen containing the freeze-dried extract at 10, 100, & 1000 U/vial + 12 × 12 mls of water for injection	11-12
E. Merck Ltd	Norisen	Aluminium-adsorbed extract of <i>D pteronyssinus</i> mite	3 multidose vials: vial 1, 2, & 3 containing 100, 1000 & 10,000 PNU/mL ^a	12

^aFurther dilution available for very sensitive patients.

Information taken from: ABPI Data Sheet Compendium, 1985,⁶⁹ and package inserts.

pany and not in IUs. With such a complex array of formulations there is, of course, no consensus as to which preparation is the drug of choice. Fortunately, since these are not interchangeable and, as yet, there is no standardization between preparation (standardization at present being confined to ensuring the batch-to-batch consistency within each product), physicians tend to use the preparation of a single manufacturer and stick to that. This is undoubtedly the safest procedure at present.

Hyposensitization to various allergens has been performed since the early part of this century.^{80,81} Conclusive results in placebo-controlled double-blind trials have been obtained for allergic rhinitis with pollen extracts and in sting sensitivity with hymenoptera venoms (for review see Fink⁸²). In the case of hyposensitization to mite, a number of controlled trials have been carried out and reviewed⁸³ and, although many have given inconclusive results, there is mounting evidence of improvement in clinical parameters in some of the trials. From 1968 to 1985, 17 placebo-controlled trials using house dust or mite extract have been reported in 19 separate papers (Table 5). These studies used either aqueous extracts, alum-precipitated, or tyrosine-adsorbed ones. Of these 17 studies, 5 demonstrated no clinical benefit from the mite therapy, 2 trials gave somewhat equivocal results, and in 8 trials mite extracts were efficacious (Table 5). In addition, 2 trials of mite extracts that are still underway promise to show benefit to the patients as judged by interim reports.^{101,102}

The different results of the trials cannot be clearly explained by the choice of preparation or duration of the injection and maintenance period. It does appear that in the more recent trials, where more stringent criteria have been applied in the selection of patients and where injections have been continued for 1 year or more, clinical improvements have been seen.

The safety of administering allergen extracts for hyposensitization is very important because not all patients will benefit from the treatment, and the disease being treated is generally not life threatening.

Immediate, serious adverse reactions to allergen extract administration such as anaphylaxis or exacerbation of asthma are well recognized, but the information on the frequency of serious adverse reactions and deaths from allergen administration is not complete. Although the safety of administering foreign antigens over a long period has been questioned due to the possibility of the formation of immune complexes and of provoking autoimmune disease or lymphoproliferative disorders, no studies have found any evidence to support these fears.^{103,104} Consequently, only the safety in the short term will be considered here.

Vervloet et al¹⁰⁵ found a low incidence (0.1%) of adverse reactions in nearly 20,000 patients in France receiving allergen extracts of different specificities, and attributed most of these reactions to physician error. A far higher incidence of 5 to 15% is reported in venom hyposensitization,¹⁰⁶ but this probably reflects not so much an inherent danger of venoms but the fact that the allergens are administered in higher doses, ie, up to 100 µg. Rands and Godfrey¹⁰⁷ described how the administration of various allergen preparations in Hampshire resulted in bronchospasm and anaphylaxis in 6.6% and 2.5% of patients, respectively.

In the United Kingdom a system for reporting adverse reactions to med-

Table 5. Controlled Trials of House Dust or House Dust Mite Hyposensitization in Asthma and Allergic Rhinitis

Treatment extract		No. of patients entered/ completed	Age (yrs)	Length of trial	Entry criteria (exclusions)
Active extract	Placebo				
House dust	Carbol saline	96/70	7+	2 yrs	Sensitivity to HD, positive SPT (corticosteroid users)
House dust (Nyegaard) House dust (ALK)	Phenol saline + color	93/80	2-14	2½-3 yrs	Asthma, positive BPT & SPT (previous hyposensitization)
Aqueous <i>D</i> <i>pteronysinus</i>	Human skin scales	22/20	11-48	4 m	Asthma &/or perennial rhinitis + positive SPT
Aqueous <i>D farinae</i> (Bencard)	House dust	34/34	16-56	6-9 m	Bronchial asthma, clinical sensitivity to house dust & positive skin test to <i>D</i> <i>farinae</i>
House dust mite	House dust	30	7-21	4 m	Perennial asthma, positive SPT (positive SPT to other allergens)
<i>D pteronyssinus</i> (Beecham)	Carbol saline	96/91	10+	1-3 m	Perennial asthma &/or rhinitis, positive SPT, positive nasal challenge (hyposensitization in last 3 yrs)
<i>D farinae</i> purified house dust		42/?	6-15	1 yr	Asthma, positive SPT to <i>D</i> <i>farinae</i> & <i>D pteronyssinus</i>
Tyrosine-adsorbed <i>D pteronyssinus</i> Migen	Tyrosine	55/45	A	1+ yr	Perennial asthma, positive SPT, clinical symptoms with house dust or nasal challenge with <i>D</i> <i>pteronysinus</i> (hyposensitization)
<i>D pteronyssinus</i> (Bencard)	Saline	72/66	>8	1½ yr	Rhinitis with or without asthma, positive nasal challenge with <i>D</i> <i>pteronysinus</i>
Pyridine-extracted, alum- precipitated <i>D</i> <i>farinae</i> Allpyral	Saline	16/14	A	15 m	Asthma, positive SPT to <i>D</i> <i>farinae</i> & <i>D pteronyssinus</i> (hyposensitization)
Tyrosine-adsorbed <i>D pteronyssinus</i> Migen (Bencard) 6 injections + 6 maintenance injections	Tyrosine	28/28	13-49	1½ yr	Perennial asthma & strong skin test response (corticosteroids)

Table 5 (continued)

Methods of assessment	Comments	Outcome (E/N)	Reference
Diaries & drug use	Advice on dust control to both groups. No significant difference between groups	N	84
Repeated BPT, diaries	Both HD extracts gave significant improvement in 90% ($p < .01$)	E	85
Diaries & drug use	10/11 had subjective- & doctor-assessed improvement on <i>D pteronyssinus</i>	E	86
Diaries & drug use	Advice on dust control given. 78% active vs 38% placebo had subjective improvement. ($p < .05$). 7/9 in active group reduced corticosteroids	E	87
Diaries, weekly ventilatory tests, drug use & clinical observations by physician w/o knowledge of treatment	House dust found superior to house dust mite—50% vs 25% showed clinical improvement	N	88
Diaries, nasal sensitivity, use of drugs, IgE & IgG levels, HR	Advice on dust control. Significant improvement in symptoms, increase in specific & total IgG, diminished HR in treatment group	E	89
Height & weight, respiratory function, examination	Improvement in height & weight in active group; but 2 groups not balanced for age, etc.	E?	90
Symptoms, spirometric values, use of drugs, SPT & nasal challenge	No significant difference in any parameter	N	91
Diary, physician's monthly assessment, SPT, nasal challenge, specific IgE & IgG	Improvement on maintenance as assessed by patient & physician	E	92
Diary, peak flow, bronchial challenge	Advice on mite control. No clinical improvement subjectively or objectively, but reduction in bronchial reactivity in active group	N	93
Diary, drug use, clinical examination	Good response in Migen + maintenance group. 5/16 Migen discontinued bronchodilators, whereas all placebo continued	E	94

(continued)

Table 5. Controlled Trials of House Dust or House Dust Mite Hyposensitization in Asthma and Allergic Rhinitis (*continued*)

Methods of assessment		Comments			Outcome (E/N)	Reference
Tyrosine-adsorbed <i>D pteronyssinus</i> Migen (Bencard)	Tyrosine	63/51	5-14	1 yr	Moderate-severe bronchial asthma tested by bronchial challenge	
Aqueous <i>D pteronyssinus</i> 3x strength 18 injections + maintenance 18 injections + placebo	Extraction medium	70/46	16-50	2 yr	Asthma, positive SPT, worsening of symptoms with HD, &/or positive NPT (seasonal asthma, symptoms with animals danders, previous hyposensitization, pregnancies)	
Tyrosine-adsorbed <i>D pteronyssinus</i> Migen (Bencard)	Tyrosine	51/51	5-14	2 yr	Participants from active or placebo groups in trial 95	
Tyrosine-adsorbed <i>D pteronyssinus</i> Migen (Bencard)	Tyrosine	39/35	17-36	1 yr +	Perennial rhinitis, positive SPT & NPT.	
Tyrosine-adsorbed <i>D pteronyssinus</i> Migen (Bencard)	Tyrosine	22/18	19-40	1 yr	Perennial asthma with or without rhinitis, SPT, BPT, NPT & specific IgE (permanent wheezing, corticosteroids)	
Tyrosine-adsorbed <i>D pteronyssinus</i> Migen (Bencard)	Tyrosine	56/51	5-15	2 yr	Perennial asthma & positive bronchial challenge	
<i>D pteronyssinus</i> Pharmalgen (Pharmacia)	Histamine	57/NG	—	1 yr	Rhinitis	
Sodium-alginate conjugated <i>D pteronyssinus</i> Conjuvac (Dome-Hollister Stier)	NG	64/57	mean 30.5	1 yr	Perennial rhinitis	

Summary total: 17 separate clinical trials—5 unfavorable/8 favorable/2 inconclusive/2 underway
 E = efficacious; N = nonefficacious; SPT = skin prick test; BPT bronchial provocation test; HD = house dust; HR = histamine release; A = adult; NPT = nasal provocation test; PEFR = peak expiratory flow rate; NG = not given.

Table 5 (*continued*)

Methods of assessment	Comments	Outcome (E/N)	Reference
Diary, drug use, PEFR recordings & 2 1-monthly examinations	Subjective improvement in asthma & rhinitis. Significant reduction in drug score in active group. Late reaction to bronchial challenge lost in $\frac{1}{2}$ of active group	E	95
Diary records, drug use & overall blind assessment by doctors	Corticosteroids used throughout trial. Only improvement of active versus placebo in night asthma score of patients not using corticosteroids. No increased benefit with maintenance	N	96
Drug use, PEFR	2nd year of Migen trial (95) Reallocation of patients to active or placebo groups on an open basis produced increased need of drugs, & deterioration in mean thoracic gas volume	E	97
Diary, NPT, SPT, total & specific IgE	More patients withdrew from placebo than active group ($p < 0.05$); symptomatic improvement greater in active group	E	98
Diary, medication score, PEFR recorded daily, total IgE, specific IgE, IgG & HR	Advice on HD control; Significant reduction in medication & PEFR score only in 1st 6m. No significant change in IgE & IgG	E	99
Diary, drug use, PEFR	cf 97 = 2nd year of Migen trial 95. Active group had reduced severity of asthma and reduced drug use	E	100
Nasal symptoms	Decrease in symptoms in active group at 3 m & in nasal sensitivity at 8 & 12m. Study still underway	E	101
Diary, nasal allergen & non-specific reactivity	Study still underway but results look promising	E?	102

ications exists and indicates approximately 1 death per year has been associated with allergen hyposensitization over the last 20 years.* Recently clinicians have noticed an increasing number (and possibly increasing frequency) of serious adverse responses to the higher doses of mite extracts.

Overall the information available to date from the clinical trials and companies shows an incidence of about 1% of serious adverse reactions.

Other Arthropod Extracts

The Panel on Review of Allergens¹⁰⁸ lists around 70 extracts of insects licensed for diagnosis or therapy. More than half of these appear suitable, or likely to be suitable, for diagnostic use and thus have been recommended to remain on the market. However, demonstration of efficacy in immunotherapy has not been shown for the majority of extracts and, for most, the small number of patients excludes any controlled trial. There have been no placebo-controlled double-blind trials for either insect or storage mite extracts reported in the literature, although individuals have been considered to show benefit following open therapy with cockroach and screwworm fly extract.^{23,29} Clinical trials amongst farmers and dock workers who have inhalant allergies to some of the storage mites that are present in the goods they handle are currently being performed (AD Blainey, personal communication).

Standardization

History

For many years the World Health Organization (WHO) has been assisting in the establishment of ISs for biologic materials. The earliest standard was produced in 1925 (insulin) and more recently for the human serum immunoglobulins IgA, IgG, and IgM (NIBSC 67/86), human serum immunoglobulin D (IgD) (NIBSC 67/37), and human serum immunoglobulin E (IgE) (NIBSC 75/502). A complete list of these is published every 2 years and includes around 200 standards.¹⁰⁹ The primary purpose of ISs is to enable the potency or activity of similar materials to be measured in IUs. As long as suitable techniques are available for the measurement of the activity, then it is not necessary or even desirable that either the standard or the preparations under test are homogeneous (with the proviso that other substances present do not interfere with this measurement).

The production of standards for allergens is complex; all naturally occurring allergens have been shown to be a mixture of proteins, glycoproteins, carbohydrates, and peptides. In each of these mixtures a number of separate components prove to be allergenic in susceptible individuals. For a considerable time this fact impeded progress towards producing standards for allergens. It was obviously unrealistic to wait until all the major allergens for each of these sources had been purified. It was therefore decided to produce standards typical of each allergen source, ie, ones that produced positive skin

*Br Med J 293 (6552):948, 1986

tests and that could be used as standards in the *in vitro* tests then being used, such as direct RAST, RAST-inhibition allergen assay, and histamine release. This material would contain in measurable amounts all of the important allergens (major allergens) that had then been characterized or purified.

It was not until 1981, when the Allergen Standardization Subcommittee of the International Union of Immunological Societies (IUIS) set out these concrete aims, that rapid progress towards allergen standardization took place. A preparation for *D pteronyssinus* mite was among the 3 allergens listed by the IUIS as being of the highest priority for standardization.¹¹⁰

Methods Used for Preparation of the D pteronyssinus International Standard

The *D pteronyssinus* extract used as the IS was chosen from 10 coded preparations in a preliminary study.³⁴ All except 2 preparations (a freeze-dried NIBSC reference reagent and an extract made from mites kindly donated by Dr. Spieksma) were obtained directly from commercial manufacturers of mite extracts. Before examination of these to assess their suitability to serve as standards, those in liquid form were dialyzed to remove phenol or glycerol and the others were reconstituted from the freeze-dried state. All were then coded, dispensed in 1-mL volumes, freeze-dried, and sealed into glass ampules under nitrogen. They were examined by 3 laboratories for biologic activity, potency, and number of characteristic antigens and allergens. Several extracts were judged suitable based on their contents of allergens and performance *in vivo* and *in vitro* assays. One of them was chosen both because of its availability and because full information was available about its growth conditions. Material from the same batch was prepared as the IS.

The extract chosen as the IS for *D pteronyssinus* was produced from mites grown on acetone-washed human skin scales and yeast in equal parts.¹¹¹ The culture used contained adult mites, larvae, nymphs, eggs, and mite feces and it was extracted in ammonium bicarbonate solution and freeze-dried from volumes of 45 mL. At NIBSC the extract was reconstituted with distilled water to a concentration of 1-mg dry wt/mL, pooled, filtered through membranes of pore size 0.22 μ m, and dispensed into 4000 glass ampules in 1-mL aliquots and freeze-dried. After secondary dessication the ampules were flushed with nitrogen and sealed by fusion of the glass neck. This method of preservation is designed to ensure uniformity of contents throughout the 4000 ampules and their stability for many years (at least 15). Uniformity of the batch was tested by assessing the wet and dry weight of the filled ampules, moisture content, and stability by *in vitro* assay of the ampule contents after various time intervals up to 3 years after storage at a range of temperatures (-20°C , $+4^{\circ}\text{C}$, $+20^{\circ}\text{C}$, $+37^{\circ}\text{C}$, and $+45^{\circ}\text{C}$).^{48,112}

The proposed IS, together with 2 other freeze-dried and 1 glycerol saline extract, was examined coded in a collaborative study by 19 laboratories in 11 countries.⁴⁸ Thirteen laboratories performed a total of 44 RAST-inhibition assays for overall potency and were able to measure the potency of the other preparations in terms of that of the IS. Seven laboratories performed CIE and CRIE in a total of 16 assays to determine both the antigenic composition and

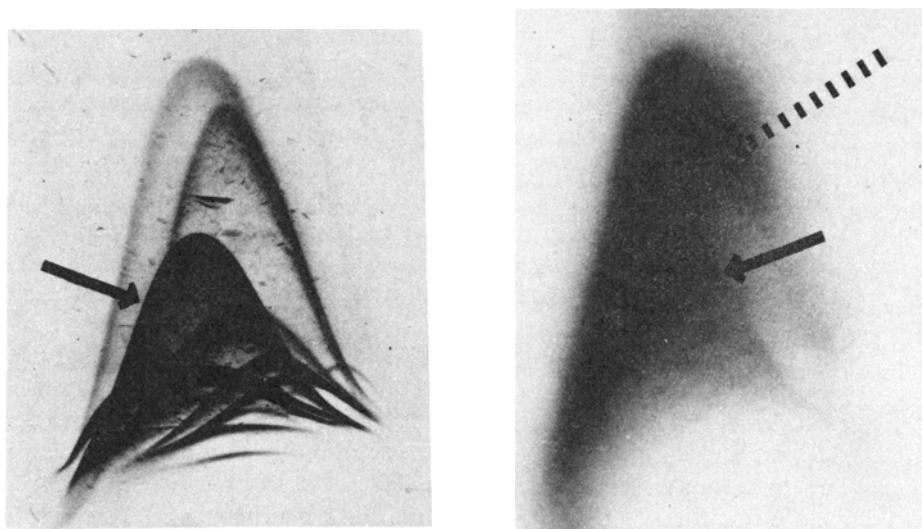
to identify the allergens present in the extracts. Figure 3 shows an example of CIE and its accompanying CRIE, and Figure 4 is a composite drawing of the precipitin arcs with the important allergens indicated. Specific allergens were measured using RIA, rocket immunoelectrophoresis (RIE), and a monoclonal antibody technique; excellent correlation was obtained. The IS was demonstrated to be a biologically and immunologically active preparation, having all of the allergens recognized to date. It contained 300 μg of protein per ampule of which about 5% was judged to be the major allergen (Der pI). A detailed report on the collaborative study was submitted to the WHO Expert Committee on Biological Standardization who in 1984 established the preparation as the first IS of *Dermatophagoides pteronyssinus* (house dust mite) extract with a unitage of 100,000 IU/ampule.¹¹³

Use of Standards

The collaborative studies to select the *D pteronyssinus* IS have led to an intensive amount of work on mite extracts. With the possibility of control authorities proposing legislation many manufacturers have been comparing the potency of their own mite extracts with that of the new standard and measuring the major allergens in terms of those in the standard. The demonstration, in the course of the preliminary study,³⁴ that a number of mite extracts contained antigens from the growth medium, most notably of horse origin, led, as we understand it, at least 1 company to seek an alternative method of culture and another to grow their own mites.

Ideally the availability of an IS should allow rapid progress towards stand-

Figure 3. A) Crossed-immunoelectrophoresis of the IS for *D pteronyssinus* (house dust mite) extract (NIBSC 82/518). The major allergen Der pI is indicated with a solid arrow. (Method as in Figure 2.) B) Crossed-radioimmuno-electrophoresis pattern of IS after incubation with serum pool of mite-allergic patients (NIBSC 82/528). The major allergens Der pI and Der pII are indicated with solid and dashed arrows, respectively.



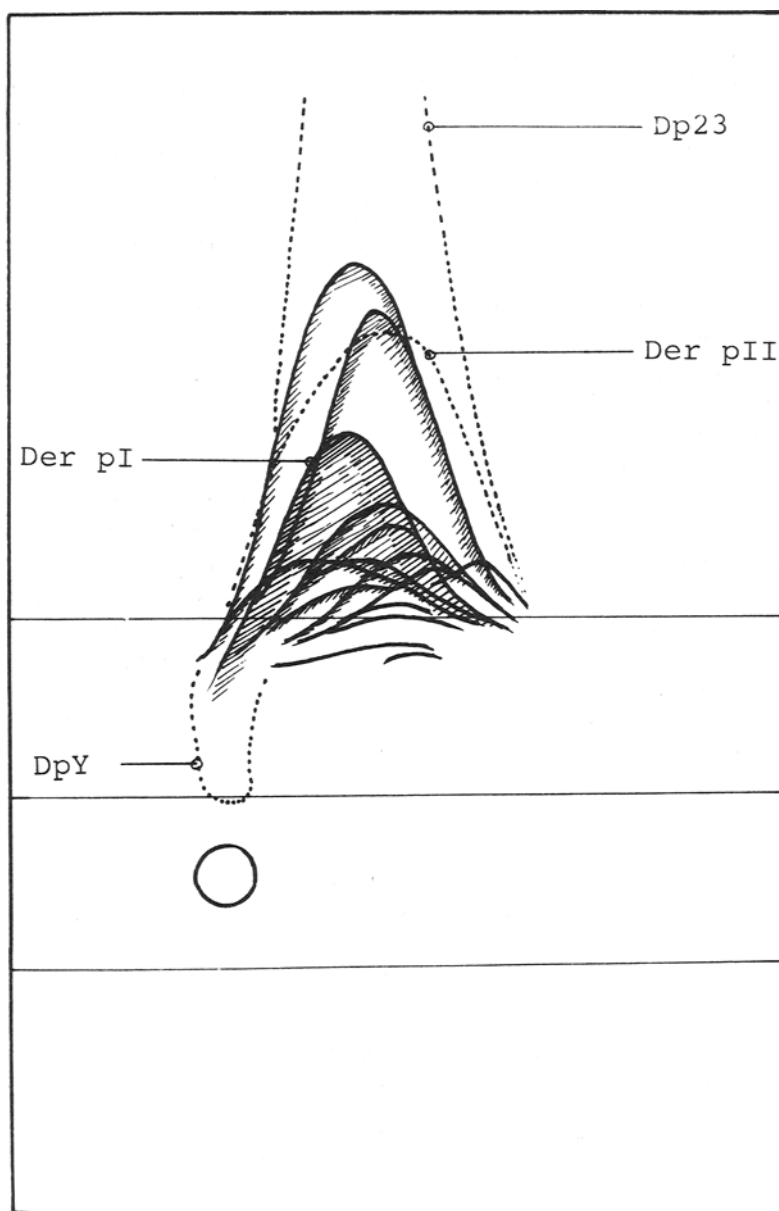


Figure 4. Reference drawing of precipitin arcs produced by CIE and CRIE of the IS for *D pteronyssinus* (house dust mite) extract (NIBSC 82/518). The allergens are labelled.

ardization of like products. For example, the potency of penicillin is routinely expressed in IU, and, more closely related to allergy, is the IU used for total serum IgE levels. Admittedly there are problems with the use of the new allergen ISs where, unlike penicillin, there is not just a single active component. Since the activity or potency of *D pteronyssinus* resides in a number of different molecules, the activity of the particular molecules being measured

depends to a large extent on the reagents and techniques used. If RAST inhibition is used as a method of potency test, then the IU assigned to a preparation will depend to a certain extent on whether the human serum pool has IgE antibodies to all, or just a few, of the antigens present. This can be overcome by the provision of a well-characterized pool of sera from individuals specifically allergic to *D pteronyssinus*, and such a pool has been prepared to accompany the IS.³⁴ Additionally, with the progress made in identifying and characterizing the important allergens, the problems are diminishing. It is possible now to measure Der pI by a variety of methods in terms of the International Standard and get a far more precise result than could be obtained for overall potency by RAST inhibition. As commercially available allergen extracts become standardized relative to the IS it should be possible to explain the differences in reported frequencies of positive skin test reactions and in results of therapy.

Standardization of commercial house dust mite extracts may well remain confined to expression of potency in IU and a maximum and minimum requirement. Owing to the complex nature of the extracts and the variation in growth media used, it is not envisaged that the existing extracts on the market would become entirely interchangeable. However, with the introduction of the *D pteronyssinus* standard (and other standards), it becomes much easier to assess different culture conditions or extraction techniques. It is possible that these studies will lead not only to a much better understanding of mite allergy, but also to general agreement about the optimal extracts for diagnostic and therapeutic use.

References

1. Nathanson ME: Arthropods and allergy. *J Asthma Res* 7(2):55–63, 1969
2. Ancona G: Asma epidemico da *Pediculoides ventricosus*. *II Policlinico Sezione Medica* 30:45–70, 1923
3. Blythe ME: Some aspects of the ecological study of the house dust mites. *Br J Dis Chest* 70:3–31, 1976
4. Wharton GW: House dust mites. *J Entomology* 12:577–621, 1976
5. Bellas TE: Insects as a cause of inhalant allergies. *A Bibliography*, 2nd edition. Division of Entomology Report No 25. Commonwealth Scientific and Industrial Research Organization, November 1982
6. Fain A: Nouvelle description de *Dermatophagoides pteronyssinus* (Troussart 1897). Importance de cet acarien en pathologie humaine (Psoroptidae: Sarcoptiformes). *Acarologia* 8:302–327, 1966
7. Voorhorst R, Spieksma M, Varekamp H, et al: The house dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces identify with the house dust allergen. *J Allergy* 39(6):325–339, 1967
8. Spieksma FThM, Spieksma-Boezeman MIA: The mite fauna of dust with particular reference to the house dust mite *Dermatophagoides pteronyssinus*. *Acarologia* 9:226–241, 1967
9. Miyamoto T, Oshima S, Mizuno K, et al: Cross-antigenicity among six species of dust mites and house dust antigens. *J Allergy* 44(4):228–238, 1969
10. Voorhorst R, Spieksma FThM, Varekamp H: House dust atopy and the house dust mite. Leiden, Stafleus Scientific Publishing Company, 1969
11. El-Heffny A, Frankland AW: House dust and mites as causes of inhalant allergic problems in the UAR, in 7th International Congress of Allergology: Abstracts of Free Communications (Excerpta Medica International Congress Series, No 211) p 3. Amsterdam, Excerpta Medica Foundation, 1970

12. Frankland AW: House dust mites and allergy. *Arch Dis Child* 47:327–329, 1972
13. Nadchatram M, Fournier G, Gordon BL, et al: House dust mites in Hawaii. *Ann Allergy* 46(4):197–200, 1981
14. Dowse GK, Turner KJ, Stewart GA, et al: The association between *Dermatophagoides* mites and the increasing prevalence of asthma in village communities within the Papua New Guinea highlands. *J Allergy Clin Immunol* 75:75–83, 1985
15. Wharton GW: Mite and commercial extracts of house dust. *Science* 167:1382, 1970
16. Korsgaard J: Mite asthma and residency: A case control study on the impact of exposure to house dust mite in dwellings. *Am Rev Resp Dis* 128:231–235, 1983
17. Goldschmidt H, Kligman AM: Quantitative estimation of keratin production by the epidermis. *Arch Dermatol* 88:709–712, 1964
18. Cuthbert OD, Brostoff J, Wraith DJ, et al: 'Barn allergy': Asthma and rhinitis due to storage mites. *Clin Allergy* 9:229–236, 1979
19. Murray AB, Ferguson AC, Morrison BJ: Sensitization to house dust mites in different climatic areas. *J Allergy Clin Immunol* 76:108–112, 1985
20. Wirtz RA: Allergic and toxic reactions to nonstinging arthropods. *Ann Rev Entomol* 29:47–69, 1984
21. Frankland AW, Lunn JA: Asthma caused by the grain weevil. *Br J Ind Med* 22:157–159, 1965
22. Frankland AW: Locust sensitivity. *Ann Allergy* 11:445–453, 1953
23. Dille JR, Gibbons HL, Spikes GA: Allergic problems in screwworm fly eradication program personnel. *Aerosp Med* 39:1116–1119, 1968
24. Yunginger JW, Jones RT, Leiferman KM, et al: Immunological and biochemical studies in beekeepers and their family members. *J Allergy Clin Immunol* 61:93–101, 1978
25. Frankland AW, Lessof MH: Allergy, insects and arachnids, in *Immunological and Clinical Aspects of Allergy*. Lancaster (UK), MTP Press, 1981, pp 373–388
26. Baur X, Ziegler D, Reichenbach-Klinke HH, et al: Detection of potent insect antigens for humans: Hemoglobins (erythrocruorins) of chironomids. *Naturwissenschaften* 67:365–366, 1980
27. Baur X, Dewair M, Fruhmann G, et al: Hyposensitivity to chironomids (non-biting midges): Localization of the antigenic determinants within certain polypeptide sequences of hemoglobins (erythrocruorins) of *Chironomus thummi thummi* (Diptera). *J Allergy Clin Immunol* 69:66–76, 1982
28. Gad El Rab MO, Thatcher DR, Kay AB: Widespread immunoglobulin E-mediated hypersensitivity in the Sudan to the green nimiti midge, *Cladotanytarsus lewisi* (Diptera Chironomidae) 2. Identification of a major allergen. *Clin Exp Immunol* 41:389–396, 1980
29. Kang B, Vellody D, Homberger H, et al: Cockroach cause of allergic asthma—its specificity and immunologic profile. *J Allergy Clin Immunol* 63:80–86, 1979
30. Hulett AC, Dockhorn RJ: House dust mite (*D. farinae*) and cockroach allergy in a Midwestern population. *Ann Allergy* 42:160–165, 1979
31. Smith MJ, Disney ME, Williams JD, et al: Clinical significance of skin reactions to mite extracts in children with asthma. *Br Med J* 2:723–726, 1969
32. Spieksma FTHM: Culture of house dust mites on animal skin scales. *Allergol Immunopathol (Madr)* 4:419–428, 1976
33. Aas K: Horse dander allergens in a house dust mite extract. *Allergy* 36:433–434, 1981
34. Ford AW, Rawle FC, Lind P, et al: Standardization of *Dermatophagoides pteronyssinus*: Assessment of potency and allergen content in ten coded extracts. *Int Arch Allergy Appl Immunol* 76:58–67, 1985
35. Tovey ER, Baldo BA: Standardization of allergens. Qualitative definition of house dust mite extracts following electroblotting and detection of components with antibody and lectin probes. *Int Arch Allergy Appl Immunol* 75:322–329, 1984
36. Kauffman HF, van der Heide S, Hovenga H, et al: Standardization of house dust mite extracts. Liberation of allergenic and antigenic components in relation to extraction conditions. *Allergy* 40:143–150, 1985
37. Tovey ER, Chapman MD, Platts-Mills TAE: Mite faeces are a major source of house dust allergens. *Nature* 289:592–593, 1981

38. Ceska M, Eriksson R, Varga JM: Radioimmuno-sorbent assay of allergens. *J Allergy Clin Immunol* 49:1-9, 1972
39. Yman L, Ponterius G, Brandt R: RAST-based allergen assay methods. International WHO-IABS Symposium on Standardization and Control of Allergens Administered to Man, Geneva 1974. *Dev Biol Stand* 29:151-165, 1975
40. Løwenstein H: Quantitative immunoelectrophoretic methods as a tool for the analysis and isolation of allergens. *Prog Allergy* 25:1-62, 1978
41. Chapman MD, Platts-Mills TAE: Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus* antigen P₁. *J Immunol* 125(2):587-592, 1980
42. Siraganian RP, Hook WA: *Manual of Clinical Immunology*, 2nd edition. Washington DC, American Society of Microbiology, 1980, p 808-821
43. Lind P, Weeke B, Løwenstein H: A reference allergen preparation of the house dust mite *D. pteronyssinus*, produced from whole mite culture—a part of DAS 76 Study. Comparison with allergen preparations from other raw materials. *Allergy* 39(4):259-274, 1984
44. Tovey ER, Baldo BA: Detection of house dust mite allergens and frequency of IgE binding following electroblotting and enzyme immunoassay. *Int Arch Allergy Appl Immunol* 76:82-85, 1985
45. Lind P: Purification and partial characterization of two major allergens from the house dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 76:753-761, 1985
46. Wahn V, Müller-Krampe B, Lind P: Activity of allergenic proteins from *Dermatophagoides pteronyssinus*. Comparison of crossed radioimmunoelectrophoresis (CRIE) and histamine release from washed leucocytes. *Allergy* 40:389-394, 1985
47. Stewart GA, Turner KJ: Physicochemical and immunochemical characterization of the allergens from the mite *Dermatophagoides pteronyssinus*. *Aust J Exp Biol Med Sci* 58:259-274, 1980
48. Ford A, Seagroatt V, Platts-Mills TAE, et al: A collaborative study on the first International Standard of *Dermatophagoides pteronyssinus* (house dust mite) extract. *J Allergy Clin Immunol* 75:676-686, 1985
49. Lind P, Løwenstein H: Identification of allergens in *Dermatophagoides pteronyssinus* mite body extract by crossed radioimmunoelectrophoresis with two different antibody pools. *Scand J Immunol* 17:263-273, 1983
50. Marsh DG, Goodfriend L, King TP, et al: Allergen nomenclature, WHO Bulletin, 64 (5):767-770, 1986
51. Chapman MD, Platts-Mills TAE: Measurement of IgG, IgA and IgE antibodies to *Dermatophagoides pteronyssinus* by antigen-binding assay, using a partially purified fraction of mite extract (F₄P₁). *Clin Exp Immunol* 34:126-136, 1978
52. Lind P: Standardization of mite extracts. Qualitative and quantitative investigations of three kinds of preparations with CRIE and RAST. *Allergy* 35:227-230, 1980
53. Lind P: Demonstration of close physicochemical similarity and partial immunochemical identity between the major allergen, Dp42, of the house dust mite, *D. pteronyssinus* and corresponding antigens of *D. farinae* (Df6) and *D. microceras* (Dm6). *Int Arch Allergy Appl Immunol* 79:60-65, 1986
54. Stewart GA: Isolation and characterization of the allergen Dpt 12 from *Dermatophagoides pteronyssinus* by chromatofocusing. *Int Arch Allergy Appl Immunol* 69:224-230, 1982
55. Le Mao J, Dandeu JP, Rabillon J, et al: Comparison of antigenic and allergenic composition of two partially purified extracts from *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* mite cultures. *J Allergy Clin Immunol* 71(6):588-596, 1983
56. Stewart GA, Turner KJ: Physicochemical and immunochemical characterization of the high molecular weight allergens from *Dermatophagoides pteronyssinus* with particular reference to the tridacrin, Con-A and S107 reactive components. *Aust J Exp Biol Med Sci* 58:275-288, 1980
57. Stewart GA, Butcher A, Turner KJ: Characterization of a very high density lipoprotein allergen, Dpt 4, from the house dust mite *Dermatophagoides pteronyssinus*. *Int Arch Allergy Appl Immunol* 71:340-345, 1983
58. Dandeu JP, Le Mao J, Lux M, et al: Antigens and allergens in *Dermatophagoides farinae* mite II: Purification of AG11, a major allergen in *Dermatophagoides farinae*. *Immunology* 46:679-687, 1982

59. Heymann PW, Chapman MD, Wilkins SR, et al: Use of monoclonal antibodies to examine cross-reactivity and species-specific epitopes on major allergens from *D. pteronyssinus* and *D. farinae*. *Ann Allergy* 55(2) Abstract, 77:244, 1985
60. Heymann PW, Chapman MD, Platts-Mills TAE: Antigen Der F1 from the dust mite *Dermatophagoides farinae*: Structural comparison with antigen P₁ from *D. pteronyssinus* and epitope specificity of murine IgG and human IgE antibodies. *J Immunol* 137:2841–2847, 1986
61. Lind P: Enzyme-linked immunosorbent assay (ELISA) for determination of major excrement allergens of the house dust mite species *D. pteronyssinus*, *D. farinae* and *D. microceras*. *Allergy* 41(6):442–451, 1986
62. Lind P, Ipsen H: Classification of epitopes on *D. pteronyssinus* allergens from three species. *Ann Allergy* 55(2) Abstract, 77:245, 1985
63. Chapman MD, Sutherland WM, Platts-Mills TAE: Recognition of two *Dermatophagoides pteronyssinus*-specific epitopes on Antigen P₁ by using monoclonal antibodies. *J Immunol* 133:2488–2495, 1984
64. Platts-Mills TAE, Heymann PW, Chapman MD, et al: Cross-reacting and species-specific determinants on a major allergen from *Dermatophagoides pteronyssinus* and a *D. farinae*: Development of a radioimmunoassay for antigen P₁ equivalent in house dust and mite extracts. *J Allergy Clin Immunol* 78:398–407, 1986
65. Arlian LG, Geis DP, Vyszynski-Moher DL, et al: Antigenic and allergenic properties of the storage mite *Tyrophagus putrescentiae*. *J Allergy Clin Immunol* 74:166–171, 1984
66. Ford AW, Topping MT, Thompson J, et al: IgE binding and antigenic relationships of storage mites by RAST inhibition, crossed immunoelectrophoresis and crossed radioimmuno-electrophoresis, in preparation
67. Arlian LG, Geis DP, Vyszynski-Moher DL, et al: Cross antigenic and allergenic properties of the house dust mite *Dermatophagoides farinae* and the storage mite *Tyrophagus putrescentiae*. *J Allergy Clin Immunol* 74:172–179, 1984
68. Tee RD, Cranston PS, Dewar M, et al: Evidence for haemoglobins as common allergenic determinants in IgE-mediated hypersensitivity to chironomid (non-biting midges). *Clin Allergy* 15:335–343, 1985
69. BPDI Data Sheet Compendium 1984–85: Datapharm Publications Ltd., 12 Whitehall, London SW1A 2DY
70. Editorial BMJ: Hyposensitization to house dust mite. *Br Med J* 280(6214):589–590, 1980
71. Maunsell K, Wraith DG, Cunningham AM: Mites and house dust allergy in bronchial asthma. *Lancet* i:1267–1270, 1968
72. Pepys J, Chan M, Hargreave FE: Mites and house dust allergy. *Lancet* i:1270–1272, 1968
73. Sarsfield JK, Gowland G, Toy R, et al: Mite sensitive asthma in childhood; trial of avoidance measures. *Arch Dis Child* 49:716–721, 1974
74. Murray AB, Ferguson AC: Dust free bedrooms in the treatment of asthmatic children with house dust mite allergy: A controlled trial. *Pediatrics* 71:418–422, 1983
75. Mulla MS, Harkrider JR, Galant SP, et al: Some house dust control measures and abundance of *Dermatophagoides* mites in Southern California (Acari:Pyroglyphidae). *J Med Ent* 12(1):5–9, 1975
76. Van de Maele P: A new strategy in the control of house dust mite allergy. *Pharmatherapeutica* 3(7):441–444, 1983
77. Platts-Mills TAE, Tovey ER, Mitchell EB, et al: Reversal of bronchial hyperreactivity during prolonged allergen avoidance. *Lancet* ii:675–678, 1982
78. Korsgaard J: Preventive measures in house dust allergy. *Am Rev Resp Dis* 125:80–84, 1982
79. Burr ML, Dean BV, Merrett TG, et al: The effects of anti-mite measures on children with mite-sensitive asthma: A controlled trial. *Thorax* 35:506–512, 1980
80. Noon L: Prophylactic inoculation against hayfever. *Lancet* i:1572–1573, 1911
81. Freeman J: Further observations on the treatment of hayfever by hypodermic inoculation of pollen vaccine. *Lancet* ii:814–817, 1911
82. Fink JN: Immunotherapy in asthma. *J Allergy Clin Immunol* 76:402–404, 1985
83. Warner JO: Hyposensitization in asthma: A review. *J R Soc Med* 74:60–65, 1981
84. British Tuberculosis Association: Treatment of house dust allergy. *Br Med J* 3:774–777, 1968

85. Aas K: Hyposensitization in house dust allergy asthma. A double blind controlled study with evaluation of the effect of bronchial sensitivity to house dust. *Acta Paediatr Scand* 60:264–268, 1971
86. Smith AP: Hyposensitization with *Dermatophagoides pteronyssinus* antigen: Trial in asthma induced by house dust. *Br Med J* 4:204–206, 1971
87. Maunsell K, Wraith DG, Hughes AM: Hyposensitization in mite asthma. *Lancet* i:967–968, 1971
88. Smith JM, Pizarro YA: Hyposensitization with extracts of *Dermatophagoides pteronyssinus* and house dust. *Clin Allergy* 2:281–283, 1972
89. D'Souza MF, Pepys J, Wells ID, et al: Hyposensitization with *Dermatophagoides pteronyssinus* in house dust allergy: A controlled study of clinical and immunological effects. *Clin Allergy* 3:177–193, 1973
90. Taylor B, Sanders SS, Norman AP: A double blind controlled trial of house mite fortified house dust vaccine in childhood asthma. *Clin Allergy* 4:35–40, 1974
91. Gaddie J, Skinner C, Palmer KNV: Hyposensitisation with house dust mite vaccine in bronchial asthma. *Br Med J* 2:561–562, 1976
92. Gabriel Sister M, Ng HK, Allan WGL, et al: Study of prolonged hyposensitization with *D. pteronyssinus* extract in allergic rhinitis. *Clin Allergy* 7:325–336, 1977
93. Newton DAG, Maberley DJ, Wilson R: House dust mite hyposensitization. *Br J Dis Chest* 72:21–28, 1978
94. Marques RA, Avila R: Results of a clinical trial with a *Dermatophagoides pteronyssinus* tyrosine adsorbed vaccine. *Allergol Immunopathol* VI:231–235, 1978
95. Warner JO, Price JE, Soothill JF, et al: Controlled trial of hyposensitization in *Dermatophagoides pteronyssinus* in children with asthma. *Lancet* ii:912–915, 1978
96. Mite Allergy Subcommittee of the Research Committee of the British Thoracic Association: A trial of house dust mite extract in bronchial asthma. *Br J Dis Chest* 73:260–270, 1979
97. Warner JO: House dust mite hyposensitization in asthma. *Proc Int Cong Allergol Clin Immunol* (9th: 1982: London) London, MacMillan, 1983, pp 301–305
98. Davies RJ, Blainey AD: Evaluation and treatment of house dust mite allergy in perennial rhinitis. *Proc Int Cong Allergol Clin Immunol* (9th: 1982: London) London, MacMillan, 1983, pp 291–295
99. Pauli G, Bessot JC, Bigot H, et al: Clinical and immunologic evaluation of tyrosine-adsorbed *Dermatophagoides pteronyssinus* extract. A double blind placebo-controlled trial. *J Allergy Clin Immunol* 74(4):524–535, 1984
100. Price JF, Warner JO, Hey EN, et al: A controlled trial of hyposensitization with adsorbed tyrosine *Dermatophagoides pteronyssinus* antigen in childhood asthma: *In vivo* aspects. *Clin Allergy* 14:209–219, 1984
101. Ewan P, Agrell B, Dreborg S: One year's immunotherapy with a standardized *D. pteronyssinus* preparation in a double-blind placebo controlled trial. *Ann Allergy* 55(2) Abstract, 345:312, 1985
102. Corrado OJ, Cresswell LA, Gajjar L, et al: Immunotherapy for perennial rhinitis with Conjuvac mite extract: A double-blind placebo controlled study. *Ann Allergy* 55(2) Abstract, 348:312, 1985
103. Levinson AI, Summers RJ, Lawley TJ, et al: Evaluation of the adverse effects of long-term hyposensitization. *J Allergy Clin Immunol* 62:109–114, 1978
104. Katelaris CH, Walls RS: A study of possible ill effects from prolonged immunotherapy in treatment of allergic diseases. *Ann Allergy* 53:257–261, 1984
105. Vervloet D, Khairallah E, Arnaud A, et al: A prospective national study of the safety of immunotherapy. *Clin Allergy* 10:59–64, 1980
106. Valentine M: Anaphylactic reactions to insects, in Goetzel EJ, Kay AB (ed): *Current Perspectives in Allergy*. Edinburgh, London, Melbourne, New York, Churchill Livingstone, 1982, pp 122–129
107. Rands DA, Godfrey RC: Side effects of desensitization for allergy—a general practice survey. *JR Coll Practitioners* 33:647–649, 1983
108. Food & Drug Administration: Panel on Review of Allergenic Extracts, Final Report. Springfield, VA, National Technical Information Service, US Dept of Commerce, 1981

109. WHO: Biological substances. International Standards, reference preparations and reference reagents, 1984. Geneva, World Health Organization, 1984
110. Geneva Documents. Geneva, February 1981. Available in Europe from H Løwenstein, The Protein Laboratory, Sigurdsgade 34, DK-2200, Copenhagen N, Denmark; outside Europe from R Goldstein, NIAID, National Institutes of Health, Westwood 755, Bethesda, MD 20205, USA
111. Voorhorst R, Spijksma FThM: Fünfzehn Jahre Hausstaubmilben—Forschung in Leiden. *Allergologie* 1(2):93–101, 1978
112. Seagroatt V, Alterman L, Ford AW: The stability of the International Standard of *Dermaphagoides pteronyssinus* (house dust mite) extract, in preparation
113. WHO Expert Committee on Biological Standardization. 35th Report. Technical Report Series 725. Geneva, WHO, 1985, p 16