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The decay of house dust mite allergens, *Der p* I and *Der p* II, under natural conditions

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Summary

Background Fluctuations in the level of mite allergens in domestic house dust are the result of changes in the balance between synthesis, removal and decay. Purely physical forces as well as enzymatic degradation, mediated by house dust inhabiting microbes, may contribute to the decay of allergens in domestic dust. Knowledge about the speed of decay is essential for an understanding of the dynamics of allergen levels.

Objective The present study is a quantitative assessment of the speed of decay at nine combinations of temperature (15°C, 20°C and 25°C) and relative humidity (33%, 55% and 75%).

Methods Samples of mite infested material of an old rug were stored at these temperature/relative humidity-combinations for 6, 12 or 18 months, after the mites were killed by either a freezing treatment or an acaricide (lindane). The microbes living in the rug presumably survive these treatments. Concentrations of *Der p* I and *Der p* II + *Der f* II, in extracts of the rug material, were measured by a radio immunoassay.

Results No significant changes in the levels of *Der p* I and *Der p* II + *Der f* II, could be detected even after 1½ year at a high temperature and humidity.

Conclusion These findings indicate that mite allergens can be extremely stable under normal domestic circumstances.

Keywords: house dust mites, *Dermatophagoides*, house dust allergens, *Der p* I, *Der p* II, *Der f* II, decay, degradation

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Introduction

In the temperate climatic regions of the world, house dust mites (*Dermatophagoides* spp.), living in carpets, mattresses and upholstered furniture, usually increase in number during the summer and show a decline during the winter [1–10]. The rising and falling mite numbers are accompanied by increasing and decreasing levels of mite allergens in house dust [11–13]. To be able to control the accumulation of allergen it is important to understand the dynamics of it. Quantitative information concerning factors controlling the synthesis of allergens by mites (temperature, humidity, food supply and population structure) and, on the other hand, the speed of removal

and decay of allergens, is a first requirement for a full understanding. The present study deals with only one factor influencing the dynamics of *Der p* I- and *Der p* II-contents of domestic house dust, namely decay.

From a medical point of view the most relevant step in the decomposition of an allergen is the alteration of epitopes, so that the molecule will no longer bind to IgE antibodies. Thus, the decay of allergens can be adequately documented using immunochemical detection methods, based on the specific affinity of antibodies for certain allergens. This decay could be a purely physico-chemical process, but enzymatic digestion by micro-organisms like fungi and bacteria may also play a role. We followed the changes in *Der p* I- and *Der p* II-contents of material cut from an old rug, during 1½ years at various combinations of temperature and relative humidity.

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Table 1. Combinations of temperature and relative humidity

Relative humidity (%)	Temperature		
	15°C	20°C	25°C
33*	a	b	c
55§	d	e	f
75‡	g	h	i

Saturated salt solutions used for achieving fixed RH were:

* $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (33%), § $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (55%), ‡ NaCl (75%).

Materials and methods

The rug

A >30-year-old woollen rug (10 mm pile) was obtained from a private home in Dronten (The Netherlands), where it was used normally until shortly before the start of the experiment. Live and dead *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* were found in a vacuum cleaner dust sample taken from the rug and analysed by a flotation technique as described previously [14].

Samples

The samples that were formed from the rug material can be divided into two groups, based on the method of killing the live mites that were present.

'Freezer-treated samples' From each of 30 widely separated sections of the rug 120 circular pieces (cross-section 10 mm) were punched. One punch from each section was put into a separate flask by random assignment, resulting in 120 flasks, each one containing a sample of 30 punches. By doing so the allergen contents of the samples are expected to approach a normal distribution. Mites were killed by freezing: 1 week at -20°C , while the flask was tightly closed.

Lindane-treated samples Another 30 sections were cut from the rug and treated with lindane for killing mites. The material was completely soaked with a commercial preparation (Lindafor FLO, rp, Rhone-poulenc), so that approximately 10 g lindane was applied to 1 m^2 of rug. After the material had dried, 40 samples of 30 punches each were formed from these sections as described above. Lindane (γ isomere of 1,2,3,4,5,6-hexachlorocyclohexanum) is a neurotoxin for insects and mites, which has been shown to be highly effective against house dust mites [15]. The compound is also known for its chemical stability [16]. We placed several mites on the treated material and observed them through a dissecting microscope. Each one of them died instantly.

Temperature, relative humidity and duration of incubation

Saturated salt solutions were used to achieve the desired relative humidities [17]. Details are listed in Table 1. After killing the mites the flasks were allowed to attain room temperature, opened and placed in a larger flask (400 cm^3)

Table 2. Numbers of samples in treatment categories

Mite killing	Incubation (months)	Temperature/humidity-combination§										Total
		—	a	b	c	d	e	f	g	h	i	
Freezing	0	4	—	—	—	—	—	—	—	—	—	4
	6	—	2	2	2	2	1	2	2	1	1	15
	12	—	3/4‡	4	4	3	4	4	4	4	4	34/35
	18	—	4	4	4	4	3	4	4	4	4	35
												88/89
Lindane	0	3	—	—	—	—	—	—	—	—	—	3
	6	—	—	—	—	—	1	—	—	—	1	2
	12	—	3	—	—	—	3	—	—	—	3	9
	18	—	3	—	—	—	3	—	—	—	3	9
												23

§ see Table 1; ‡ 3 *Der p* I- and 4 *Der p* II-values.

Table 3. *P*-values from two-factor ANOVA performed on four groups of data

Allergen	Mite killing	Factor		
		°C/RH combination	6/12/18 months	Interaction
<i>Der p</i> I	Freezing	0.89	0.80	0.998
<i>Der p</i> I	Lindane	0.63	0.12	0.40
<i>Der p</i> II	Freezing	0.61	0.13	0.44
<i>Der p</i> II	Lindane	0.60	0.53	0.84

containing an amount of saturated salt solution on the bottom. These were tightly closed and placed in a climatic room at either 15°C, 20°C or 25°C. After 6, 12 or 18 months the flasks were removed and temporarily stored at -20°C, before allergen quantification was performed.

Extraction of rug punches

Rug punches were transferred to 50 mL tubes (Nunc, InterMed, Denmark), and 20 mL of extraction buffer (PBS/0.1% Tween-20 (v/v)/0.1% NaN₃ (w/v)) was added. After overnight rotating incubation, the incubated punches were put into a syringe holder (Sherwood Medical Monoject Syringes), and centrifuged three times at 2000 rpm, resulting in a separation of the rug material and the extraction buffer. Buffer material was pooled, and the amount of *Der p* I and *Der p* II determined. Allergen contents were expressed as ng per gram rug material.

Der p I

The amount of *Der p* I in the eluates was measured in an inhibition assay as described by Chapman *et al.* [18] with some modifications: 0.05 mL of rabbit anti-*Der p* I was added to 0.05 mL of unknown sample (0.5 in case of very diluted samples) and 0.05 mL of PBS-AT (PBS/0.3% BSA [w/v] 0.1% Tween 20 [v/v]); this mixture was then incubated for 2 h on a shaking apparatus. Protein A Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) was used as solid phase (0.5 mg of Sepharose in 0.25 mL PBS-AT).

Then another 0.25 mL of PBS-AT and 0.05 mL of ¹²⁵I radiolabelled purified *Der p* I were added. After an overnight rotating incubation step, and removal of unbound components by washing, the amount of bound radiolabel was determined and *Der p* I levels were read from a calibration curve consisting of serial twofold dilutions of mite extract (Commonwealth Serum Laboratories), containing known amounts of *Der p* I and *Der p* II.

Der p II

Der p II was measured as follows: 0.05 mL mixture of two patients sera, IgE-positive to *Der p* II, and PBS-AT (1:1:2) was added to 0.5 mL of unknown sample, and incubated for 2 h on a shaking apparatus. Then Sepharose-bound monoclonal antibody to *Der p* II (0.25 mL, 2 mg MoAb-Sepharose/mL PBS-AT) and 0.25 mL PBS-AT were added, followed by overnight rotating incubation. After washing, 0.25 mL mixture of bovine serum and sheep serum (resp 3 and 0.3% in PBS-AT) and 15 000 cpm of ¹²⁵I radiolabelled anti-human IgE (sheep) in 0.2 mL PBS-AT were added, followed by a second overnight incubation. Unbound radiolabel was removed by washing, and *Der p* II levels determined by using serial twofold dilutions of mite extract as calibration curve.

Statistical evaluation

Forty-eight samples were used for preliminary observations and trials. An overview of the 89 'freezer-treated samples' and 23 'lindane-treated samples', which were included in the final analyses, is given in Table 2. The distribution of allergen concentration values was studied after a correction for treatment effects was applied. Dixon's test ($\alpha = 0.10$, one-sided, [19]) revealed only one obvious outlier among the *Der p* I-values. This value was excluded from further analyses. The distribution of the 111 remaining *Der p* I-values and 112 *Der p* II-values conformed reasonably well to a normal distribution ($SD = 2.473$ and 2.182 ng/g respectively) as judged by χ^2 analysis, so that transformation was not necessary. Analysis of variance (ANOVA) was applied to analyse the data.

Results and discussion

Der p I vs *Der p* II

Der p I- and *Der p* II-values exhibit a clear, positive correlation (Fig. 1, $r^2 = 0.32$, $n = 111$).

Fig. 1. Relation between the concentrations of *Der p* I and *Der p* II in samples.

Lindane-treatment vs freezing

The 23 lindane-treated samples were compared with the 28 (*Der p* I) or 29 (*Der p* II) corresponding samples in which the mites were killed by freezing (Table 2). The *Der p* I levels were systematically higher in the lindane-

treated samples (two-factor ANOVA, effect of mite killing method: $P = 0.005$). We cannot be sure about the true explanation for this. The possibility that, in a number of samples, mites were surviving and producing allergen seems unlikely. This could possibly happen in the freezer-treated samples but is highly unlikely in the lindane-treated samples. Indeed, the allergen levels in the freezer-treated samples, but not in the lindane-treated samples, show a slight increase with time (Fig. 2). However, the amounts of *Der p* I found in the freezer-treated samples are generally lower than in the lindane-treated samples, which contradicts this explanation. It is more likely that certain components of the lindane formulation influenced the immunochemical detection of allergens. The effect is rather small. On average, the 28 freezer-treated samples contained 14.6 ng/g *Der p* I, whereas the 23 lindane-treated samples contained 16.0 ng/g. Moreover, there is no reason to believe that this effect will have obscured differences in *Der p* I-content caused by the treatments. There is no significant interaction ($P = 0.11$) between mite killing mechanisms (freezing/lindane) and, on the other hand, treatment (temperature, relative humidity

Fig. 2. Changes in *Der p* I- and *Der p* II-concentrations with time. (a) *Der p* I (ng/g), freezer-treated samples. (b) *Der p* I (ng/g), lindane-treated samples. (c) *Der p* II (ng/g), freezer-treated samples. (d) *Der p* II (ng/g), lindane-treated samples.

and incubation time). For *Der p II* there were no statistically significant differences between the lindane-treated and the freezer-treated samples ($P = 0.17$, interaction with treatments: $P = 0.44$).

The effect of temperature, relative humidity and time

To examine the effect of temperature, relative humidity and incubation time, the lindane-treated and freezer-treated samples were considered separately. The contents (ng/g) of *Der p I* and *Der p II* after 6, 12 and 18 months of exposure to various temperatures and humidities are depicted in Fig. 2. A two-factor ANOVA revealed no statistically significant differences due to temperature and RH (Table 3). The *Der p I* and *Der p II* levels of the freezer-treated samples show a slight and statistically not significant increase with time. The allergen levels in the lindane treated samples show a slight decrease with time, especially in the case of *Der p II* ($r^2 = 0.19$, $n = 23$). This may be no more than a chance effect that works out the same for *Der p I* and *Der p II*, because the amounts of allergen in the samples are correlated (Fig. 1). Even if the decrease in the lindane-treated samples does reflect a decomposition process, the more important finding is, that this decomposition proceeds very slowly. In a perspective of allergen management a decomposition rate of this magnitude is certainly irrelevant.

Other rugs may have a different micro-flora with microbes that break down proteins more efficiently. Therefore it may be asked whether the stability of allergens as we observed it is generally true. There is some circumstantial evidence that it is. Kort and Kniest [20] studied the breakdown of *Der p I* in three vacuum cleaner dust samples at 75% RH and either 5°C or 25°C. Mites were killed by a heat treatment (60°C during 24 h). According to the authors this treatment allows the continuation of some fungal activity. Yet the *Der p I* levels after 4 years of incubation were still high and not significantly different from the initial values. Also, the cat allergen *Fel d I* in vacuum cleaner dust bags, where the possibility of cat allergen synthesis can be ruled out, showed no sign of decay after many months (S.O., Stapel, pers. comm.).

Thus, it seems that some allergens in homes are very stable. If this is generally true than the seasonal decrease of dust mite allergen levels that is usually observed in winter must be the result of removal. Much allergen is certainly removed through vacuum cleaning. For the immediate reduction of the allergen load in a home the performance of vacuum cleaners was found to be disappointing [22–27]. The time needed to remove only half of the allergen by vacuuming is distressingly long. Yet the gradual decrease in allergen content of house dust,

that is observed in the fall and early winter, must be due mainly to the removal of it through vacuuming.

House dust mite allergen levels vary enormously between houses even within a small area [7,28,29, R.T. van Strien *et al.* manuscript in preparation]. The existence of homes with very low levels of allergens next to ones with very high levels offers hope that it must be possible to accomplish radical allergen reduction by appropriate actions. Some knowledge about the factors that, directly or indirectly, influence the development of mite populations and allergen reservoirs in a home is available. This knowledge mainly comes from the observation of correlations between house dust mite density and factors like temperature and humidity and from *in vitro* studies like the present one. However, our understanding of the dynamics of allergen levels in a house is still very poor. Many factors play a role which not only change continuously, but also interact with each other. Simulation models would be indispensable to keep track of all these changes. Critical factors and optimum timing of control actions could be identified with more precision using such models. The construction of a simulation model is never easy, but the ecosystem of house dust is relatively simple. The decay of dust mite allergens could have been a factor to be taken into consideration, but the present study indicates that the decomposition process is slow enough it ignore it, even at high temperatures and humidity.

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