

# Quantitation of major allergens in dust samples from urban populations collected in different seasons in two climatic areas of the Basque region (Spain)

Echechipía S, Ventas P, Audicana M, Urrutia I, Gastaminza G, Polo F, Fernández de Corres L. Quantitation of major allergens in dust samples from urban populations collected in different seasons in two climatic areas of the Basque region (Spain).

Allergy 1995; 50: 478–482. © Munksgaard 1995.

We present the results of allergen content evaluation in 80 dust samples from 31 homes of atopic patients from two climatic areas (humid and subhumid), collected in two seasons of the year (autumn and winter). Monoclonal antibody-based immunoassays were used to quantify Der p 1, Der f 1, Der 2, Lep d 1, and Fel d 1. The results were compared according to climate, season, and the type of sensitization (Pyroglyphidae mites, storage mites, or grass pollens). We underline the predominance of *Dermatophagoides pteronyssinus* (89% of samples) over *D. farinae* (16% of samples) in our environment. Der p 1 rates were higher in the humid area (Mann-Whitney  $P < 0.001$ ), especially in the autumn (Wilcoxon  $P < 0.05$ ). Lep d 1 was detected in 23% of samples and Lep d 1 levels were higher in the homes of patients sensitized to storage mites (Mann-Whitney  $P < 0.05$ ), whereas this allergen was not detected in the homes of pollen-allergic patients. Fel d 1 was detected in nine of the 31 homes (16% of samples) although there was a cat in only one home.

S. Echechipía<sup>1</sup>, P. Ventas<sup>2</sup>,  
M. Audicana<sup>1</sup>, I. Urrutia<sup>1</sup>,  
G. Gastaminza<sup>1</sup>, F. Polo<sup>2</sup>,  
L. Fernández de Corres<sup>1</sup>

<sup>1</sup> Servicio de Alergia, Hospital Santiago Apóstol, Vitoria-Gasteiz; <sup>2</sup> Alergia e Inmunología Abelló SA, Madrid, Spain

Key words: allergenic exposure; cat; house-dust mite; major allergens; monoclonal antibodies; storage mites.

Susana Echechipía Madoz  
Sección de Alergología, CS Conde Oliveto  
Pza. de la Paz s/n  
31002 Pamplona  
Spain

Accepted for publication 8 March 1995

For more than 20 years, the mites of the Pyroglyphidae family have been considered to be the main allergenic source in house dust (36). However, other potent allergens occur in dust such as those of the dander of pet animals (2, 14, 18), molds (7), cockroach (24), and other mite families (3, 12).

It has been recently considered that exposure to high levels of allergens entails a risk of developing sensitization or of inducing asthma attacks in sensitive subjects (13, 19–22); therefore, the evaluation and control of allergen levels in homes could provide useful information in the prevention, diagnosis, and treatment of rhinitis and bronchial asthma (20).

In a rapid and precise way, the use of immunochemical techniques has enabled the estimation of the content of different allergens present in a sample of house dust; therefore, immunoassays have been used to evaluate exposure to mite and animal dander allergens (15). Recently, the production of monoclonal antibodies against groups 1 and 2 allergens (Der p 1, Der f 1, Der 2) of *Dermatophagoides pteronyssinus* and *D. farinae*, as well as against Fel d 1

of *Felis domesticus* and Lep d 1 of *Lepidoglyphus destructor* (32, 34), has enabled the evaluation of the presence of these allergens in house-dust samples through assays that are easy to perform, reproducible, and of high sensitivity and specificity (4, 15, 33, 34).

In our climate, dust mites are the main cause of respiratory allergy (26). The purpose of this work was to determine the exposure levels of our patients to different mites and cat dander, and to compare them according to climatic and seasonal changes.

## Material and methods

### Dust samples

Eighty house-dust samples, collected during two winters (March 1990 and 1991) and one autumn (September 1990) from 31 urban homes of three groups of atopic patients, were studied:

- 1) 10 patients sensitized exclusively to Pyroglyphidae mites

- 2) 11 patients sensitized to storage mites with/without sensitization to Pyroglyphidae mites
- 3) 10 patients sensitized exclusively to grass pollen who were used as a control group.

We selected homes of patients with symptoms of seasonal or perennial rhinitis and/or bronchial asthma with positive prick tests (6) and conjunctival provocation tests (17), using standardized extracts with a strength of 100 BU/ml (Alergia e Inmunología Abelló SA), and with serum specific IgE levels greater than or equal to class 3 (RAST Phadebas Pharmacia, Uppsala, Sweden) to grass pollen or mites, respectively.

Half the samples came from a humid area (precipitations of 900–1200 mm/year, from the Alto Deva area, Guipúzcoa, Spain) and the rest from a subhumid area (precipitations of 600–900 mm/year, Vitoria, Álava, Spain).

The samples were collected with a vacuum cleaner (2 min/m<sup>2</sup>), after a new bag had been placed in it. Dust was collected from the patient's bedroom including the mattress, and the living room, mainly the carpeting and other soft furnishings. The patients were cautioned not to clean these areas for 3 days before the collection of the dust. Each sample was hermetically closed and stored at 4°C until it was processed.

#### Preparation of the house-dust samples

Samples were sieved through a 300-µm mesh screen. Three grams of sieved dust was extracted in 20 ml phosphate-buffered saline (15%, w/v) with magnetic stirring for 90 min at 4°C. Afterward, the mixture was centrifuged at 22 000 g for 5 min, and the supernatant filtered through 0.45-µm filters (Millipore Flow Rate, Flow Laboratories, Germany) and stored in aliquots at –20°C.

#### Determination of major allergen content

Der p 1, Der f 1, Lep d 1, and Fel d 1 were determined by specific enzyme immunoassays with specific monoclonal antibodies bound to a solid phase, specific rabbit antiserum to each allergen, and goat antibody rabbit anti-IgG coupled to peroxidase (33, 34). Der 2 was determined by the method of Heymann et al. (9).

Allergen levels were expressed in µg/g of dust and were classified according to the score proposed by Platts-Mills et al. for group 1 *Dermatophagoides* allergens (21), which was extended to the rest of the allergens: negative (<0.05 µg/g), low (0.05–0.20), moderate (0.21–2.00), high (2.01–10), very high (>10 µg/g).

Five extreme values from five samples were eliminated. The statistical methods used were descriptive

study, correlation coefficient, chi-square, and Mann-Whitney and Wilcoxon tests for nonparametric data.

## Results

Der p 1 was detected in 89% of the samples, with moderate rates in 37%, high in 31%, and very high in 18% of the samples (Table 1). Der 2 values were correlated with those of Der p 1 ( $r=0.9$ ;  $P<0.05$ ) and Der p 1 plus Der f 1 ( $r=0.89$ ;  $P<0.05$ ). Der f 1 was detected in only 16% of the samples, while Lep d 1 was detected in 23%, with low rates in 10%, moderate in 6%, high in 3%, and very high in 4% (Table 1). Moderate-high Fel d 1 values appeared in only 16% of the samples (Table 1). In all, this was detected in only nine of the 31 homes, in spite of the fact that in eight homes there was no cat either before or during the study.

The mean level of Der p 1 in samples of the humid area was higher than in the subhumid area (Mann-Whitney,  $P<0.001$ ) (Fig. 1). No differences were found in Lep d 1 content when samples from different climatic areas were compared (Fig. 1). Most of the samples with Der p 1 and Der 2 levels higher than 2 µg/g of dust were found in the humid area (chi-square test,  $P<0.001$ ) (Fig. 2). Der p 1 and Der 2 content in the autumn of 1990 was greater than in

Table 1. Percentage of samples with undetectable (<0.05), low (0.05–0.20), moderate (0.21–2.00), high (2.01–10.00), and very high (>10 µg/g of dust) levels of Der p 1, Der f 1, Der 2, Lep d 1, and Fel d 1 allergens

|            | Der p 1 | Der f 1 | Der 2 | Lep d 1 | Fel d 1 |
|------------|---------|---------|-------|---------|---------|
| <0.05      | 11      | 84      | 4     | 77      | 84      |
| 0.05–0.20  | 3       | 0       | 1     | 10      | 7       |
| 0.21–2.00  | 37      | 12      | 39    | 6       | 6       |
| 2.01–10.00 | 31      | 4       | 40    | 3       | 3       |
| >10        | 18      | 0       | 16    | 4       | 0       |
| Detectable | 89      | 16      | 96    | 23      | 16      |

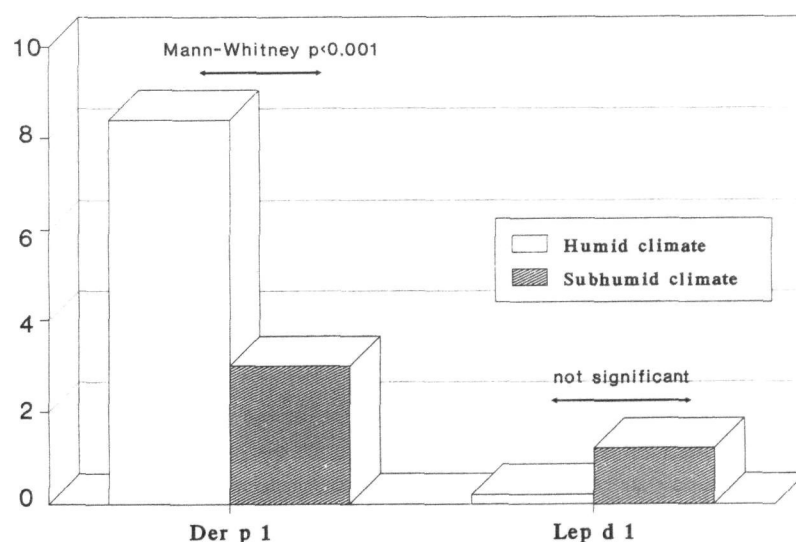


Fig. 1. Mean levels (µg/g of dust) of Der p 1 and Lep d 1 in humid and subhumid areas.

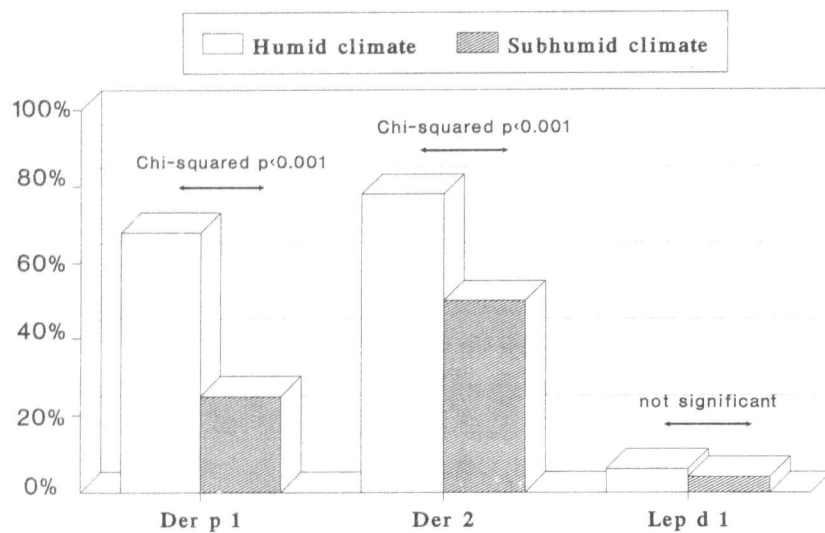


Fig. 2. Percentage of samples whose Der p 1, Der 2, and Lep d 1 content was  $> 2 \mu\text{g/g}$  of dust in humid and subhumid areas.

the two winters in the humid area (Wilcoxon  $P < 0.05$ ) (Fig. 3), while there was no seasonal difference in the subhumid area. No differences were found in Lep d 1 content when samples gathered in different seasons of the year were compared (Fig. 3).

Der p 1 content in the homes of patients sensitized to mites was not significantly different from the content in the homes of those sensitized to grass pollens; even the mean rates were higher in the homes of pollen-allergic patients than in the homes of patients allergic to Pyroglyphidae mites (Fig. 4). High values of Lep d 1 were obtained only in the homes of patients sensitized to storage mites (Mann-Whitney,  $P < 0.05$ ), and Lep d 1 was not detected in the homes of pollen-allergic patients (Fig. 4).

## Discussion

There are various methods of evaluating allergen levels in house dust (19). Of all these methods, the great advance of immunoassays with monoclonal antibodies over other immunoassays (such as RAST

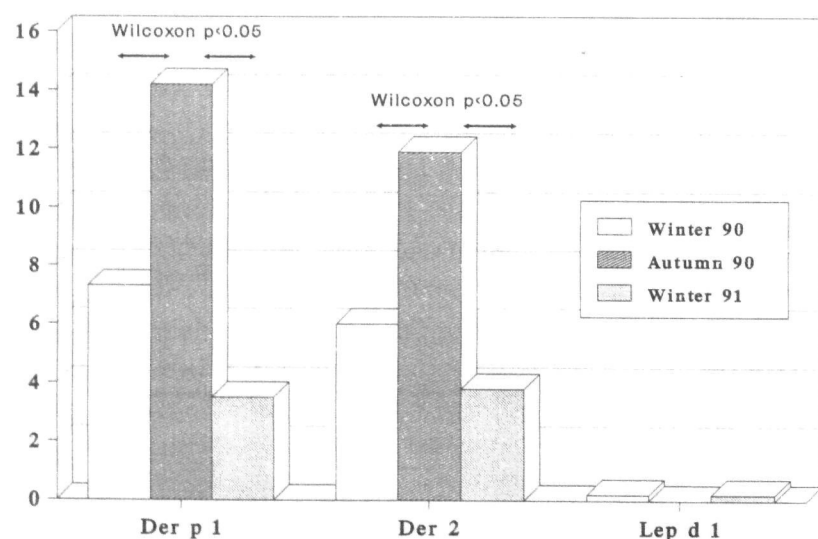


Fig. 3. Mean level ( $\mu\text{g/g}$  of dust) of Der p 1, Der 2, and Lep d 1 in winters of 1990 and 1991 and in autumn of 1990, in humid area.

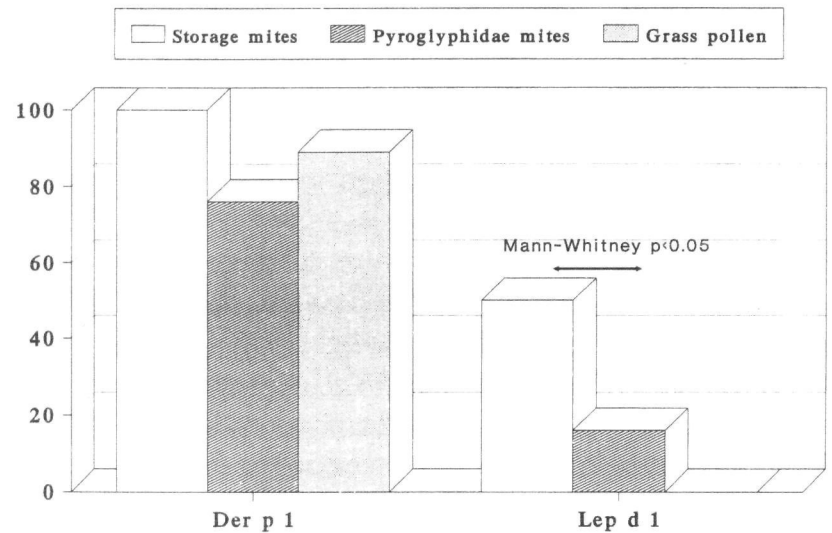


Fig. 4. Percentage of samples whose Der p 1 and Lep d 1 levels were  $> 0.05 \mu\text{g/g}$  of dust in homes of patients sensitized to storage mites, Pyroglyphidae mites, and grass pollen.

inhibition) has been highlighted, because the technique that uses monoclonal antibodies against major allergens is more sensitive, specific, and reproducible (5). In this study, we used a sandwich ELISA with monoclonal antibodies against Der p 1, Der f 1, Der 2, Lep d 1, and Fel d 1 (33, 34) to quantify the content of these allergens in the house-dust extracts from our patients' homes.

We found *D. pteronyssinus* to be predominant over *D. farinae*, in concordance with other European studies (25, 28, 37), but unlike estimations in some North American papers (27, 39). This result is probably related to the climate of our area; we do not have long dry winters, the climatic pattern that seems to favor *D. farinae* predominance (20).

We detected Lep d 1 in 23% of the samples, an important figure if one considers that the samples come from urban homes. Most of the studies on storage-mite sensitization relate to farming populations, exposed professionally to these mites in grain stores. In this group, the clinical importance of this sensitization has been shown with challenge tests (30). In some studies on urban populations, the finding of a high sensitization prevalence to these mites in patients also sensitive to *D. pteronyssinus* has been attributed to cross-reactivity between storage mites and *D. pteronyssinus* (16).

There is controversy (1, 8, 10, 31) about the *in vitro* studies on cross-reactivity. In a recent study, Johansson et al. (11) showed, through immunoblotting inhibition, that only two *L. destructor* minor components had some cross-reactivity with *D. pteronyssinus*. Polo et al. (23), by means of competition immunoassays, concluded that there is no cross-reactivity. Ventas et al. (35), in their work on Lep d 1 purification and characterization, found that this major allergen is not responsible for allergenic cross-reactivity between *L. destructor* and *D. pteronyssinus*. We found Lep d 1 levels in the homes of

patients with specific IgE against storage and Pyroglyphidae mites to be significantly higher than those found in the homes of patients sensitized only to the latter. Therefore, if Lep d 1 is species specific, our *L. destructor* patients are those most exposed to *L. destructor*. Most probably this has to do with double sensitization, and not with cross-reactivity.

On comparison of Der p 1 and Lep d 1 allergen levels, according to the source area (humid or sub-humid) and the season (autumn and winter) when the samples were collected, a different behavior for both was observed. There were significant variations of Der p 1 only in the humid area, with higher levels in autumn (Fig. 3). However, our study found no differences in Lep d 1 content between samples obtained in different areas and seasons of the year. Storage mite growth has been related to very humid homes (40). Indoor humidity levels correlate well with outdoor humidity values although absolute outdoor humidity has been reported to present higher variability than indoor humidity (21). The relationship between Lep d 1 levels and changes of indoor humidity has yet to be determined.

As for the control group made up of pollen-allergic patients not sensitized to mites, the mean content of Der p 1 in their homes did not differ from that obtained in the homes of patients sensitized to mites, a finding which is in keeping with previous results (28). However, Lep d 1 was not detected in the homes of pollen-allergic patients (Fig. 4). Given the limitations of the sample size, one might assume that exposure to *L. destructor* entails greater risk of sensitization than exposure to *D. pteronyssinus*. It would be necessary to carry out studies with a greater sample size to establish which Lep d 1 concentration favors the development of *L. destructor* sensitization.

It has been suggested that Fel d 1 quantities in house dust could be several times higher than those of mite allergens (5); in some studies, this allergen has been detected in all or almost all the samples (29, 39). This fact has not been corroborated in our study, as Fel d 1 was detected in only 16% of the samples (nine out of 31 homes). Probably, in our region, the presence of pet animals in homes is not as common as in other countries. In the only home in which the presence of a cat was confirmed during the study, Fel d 1 values varied from the highest (8.14 µg/g of dust) to being undetectable. Wentz et al. (38) have shown that cat allergen shedding varies during the day and between days, a finding which could explain our results. There was no cat before or during the collection of samples in the remaining homes, and very variable values were also obtained.

In conclusion, the most common allergen in our environment was that of *D. pteronyssinus* (89% of the samples). We detected *L. destructor* in 23% of the samples; therefore, we consider that this mite is

not a minor mite in our experience, at least if compared with *D. farinae* (16% of the samples), which is classically considered a major mite. We therefore consider that, in our environment, *L. destructor* should be considered in the diagnosis of patients with perennial rhinitis and/or bronchial asthma.

## Acknowledgment

This study was supported by grant 90/0051 from the Fondo de Investigación Sanitaria (FIS), Spain.

## References

1. ARLIAN LG, GEIS DP, VYSZENSKI-MOHER DL, BERNSTEIN IL, GALLAGHER JS. Cross-antigenic and allergenic properties of the house dust mite *Dermatophagoides farinae* and the storage mite *Tyrophagus putrescentiae*. *J Allergy Clin Immunol* 1984; 74: 172–9.
2. CARLSEN S, WEEKW B, LØWENSTEIN H. Analysis of antigens in a commercial house dust extract by means of quantitative immunoelectrophoresis. *Allergy* 1979; 34: 155–65.
3. CARSWELL F. State of the art: mites and human allergy. *Immunology* 1988; 65: 497–500.
4. CARSWELL F. State of the art: mites and human allergy. *Immunology* 1988; 65: 497–500.
5. CHAPMAN MD, HEYMANN PW, WILKINS SR, BROWN MJ, PLATTS-MILLS TAE. Monoclonal immunoassays for major dust mite (*Dermatophagoides*) allergens, *Der p* I and *Der f* I, and quantitative analysis of the allergen content of mite and house dust extracts. *J Allergy Clin Immunol* 1987; 80: 184–94.
6. CHAPMAN MD. Allergen specific monoclonal antibodies: new tools for the management of allergic disease. *Allergy* 1988; 43 (Suppl. 5): 7–14.
7. DREBORG S. Skin tests used in type I allergy skin testing. Position paper of the EAACI. *Allergy* 1989; 44 (Suppl. 10): 38–46.
8. GRAVESON S. Identification and prevalence of culturable mesophilic microfungi in house dust from 100 Danish homes. *Allergy* 1978; 33: 268–72.
9. GRIFFIN P, FORD AW, ALTERMAN L, et al. Allergenic and antigenic relationship between three species of storage mite and the house dust mite, *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1989; 84: 108–17.
10. HEYMANN PW, CHAPMAN MD, AALBERSE RC, FOX JW, PLATTS-MILLS TAE. Antigenic and structural analysis of group II allergens (*Der f* II and *Der p* II) from house dust mites (*Dermatophagoides* spp). *J Allergy Clin Immunol* 1989; 83: 1055–67.
11. JOHANSSON E, VAN HAGE-HAMSTEN M, JOHANSSON SGO. Demonstration of allergen components in the storage mite *Lepidoglyphus destructor* by an immunoblotting technique. *Int Arch Allergy Appl Immunol* 1988; 85: 8–13.
12. JOHANSSON E, BORGA A, JOHANSSON SGO, VAN HAGE-HAMSTEN M. Immunoblot multi-allergen inhibition studies of allergenic cross-reactivity of the dust mites *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 1991; 21: 511–18.
13. KORSGAARD J, DAHL R, IVERSEN M, HALLAS T. Storage mites as a cause of bronchial asthma in Denmark. *Allergol Immunopathol* 1985; 13: 143–9.
14. LAU S, FALKENHORST G, WEBER A, et al. High mite-allergen exposure increases the risk of sensitization in atopic children and young adults. *J Allergy Clin Immunol* 1989; 84: 718–25.
15. LØWENSTEIN H, GRAFF-LONNEVIG V, HEDLIN G. Immunotherapy with cat and dog dander extracts. *J Allergy Clin Immunol* 1986; 77: 497–505.

15. LUCZYNSKA CM, ARRUDA LK, PLATTS-MILLS TAE, MILLER ID, LOPEZ M, CHAPMAN MD. A two-site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp. allergens *Der p* I and *Der f* I. *J Immunol Methods* 1989; 118: 227-35.
16. LUCZYNSKA CM, GRIFFIN P, DAVIES RJ, TOPPING MD. Prevalence of specific IgE to storage mites (*A. siro*, *L. destructor* and *T. longior*) in an urban population and cross-reactivity with the house dust mite (*D. pteronyssinus*). *Clin Exp Allergy* 1990; 20: 403-6.
17. MÖLLER C, BJÖRKSTÉN B, NILSSON G, DREBORG S. The precision of the conjunctival provocation test. *Allergy* 1984; 39: 37-41.
18. OHMAN JL, LORUSSO JR, LEWIS SJ. Cat allergen content of commercial house dust extracts: comparison with dust extracts from cat-containing environment. *J Allergy Clin Immunol* 1987; 79: 955-9.
19. PLATTS-MILLS TAE, DE WECK AL. Dust mite allergens and asthma - a worldwide problem. *J Allergy Clin Immunol* 1989; 83: 416-27.
20. PLATTS-MILLS TAE, CHAPMAN MD. Dust mites: immunology, allergic disease and environmental control. *J Allergy Clin Immunol* 1987; 80: 755-75.
21. PLATTS-MILLS TAE, HAYDEN ML, CHAPMAN MD, WILKINS SR. Seasonal variation in dust mite and grass-pollen allergens in dust from the houses of patients with asthma. *J Allergy Clin Immunol* 1987; 79: 781-91.
22. POLLART SM, PLATTS-MILLS TAE. Mites and mite allergy as risk factors for asthma. *Ann Allergy* 1989; 63: 364-5.
23. POLO F, VENTAS P, CARREIRA J. Reactividad cruzada entre *Lepidoglyphus destructor* y otras especies de ácaros [Abstract]. *Rev Esp Alergol Inmunol Clin* 1992; 7 (suppl. 2): 34.
24. SCHOU C, FERNÁNDEZ-CALDAS E, LOCKEY RF, LØWENSTEIN H. Environmental assay for cockroach allergens. *J Allergy Clin Immunol* 1991; 87: 828-34.
25. SCHWARTZ B, LIND P, LØWENSTEIN H. Level of indoor allergens in dust from homes of allergic and non-allergic individuals. *Int Arch Allergy Appl Immunol* 1977; 82: 447-9.
26. Sociedad Española de Alergología e Inmunología Clínica, Alergia e Inmunología Abelló SA. Mapa epidemiológico de alergia en España: Alergológica 92. Resultados Regionales Norte. Ed. NILO Industrias Gráficas. Madrid: 1994.
27. SWANSON MC, CAMPBELL AR, KLAUCK MJ, REED CE. Correlations between levels of mite and cat allergens in settled and airborne dust. *J Allergy Clin Immunol* 1989; 83: 776-83.
28. TOVEY ER, CHAPMAN MD, WELLS CW, PLATTS-MILLS TAE. The distribution of dust mite allergen in the houses of patients with asthma. *Am Rev Respir Dis* 1981; 124: 630-5.
29. VAN DER BREMPT X, CHARPIN D, HADDI E, DA MATA P. Cat removal and *Fel d* I levels in mattresses. *J Allergy Clin Immunol* 1991; 87 (2): 595-6.
30. VAN HAGE-HAMSTEN M, IHRE E, ZETTERSTRÖM O, JOHANSSON SGO. Bronchial provocation studies in farmers with positive RAST to the storage mite *Lepidoglyphus destructor*. *Allergy* 1988; 43: 545-51.
31. VAN HAGE-HAMSTEN M, JOHANSSON SGO, JOHANSSON E, WIREN A. Lack of allergenic cross-reactivity between storage mites and *Dermatophagoides pteronyssinus*. *Clin Allergy* 1987; 17: 23-31.
32. VENTAS P, CARREIRA J, POLO F. Identification of IgE-binding proteins from *Lepidoglyphus destructor* and production of monoclonal antibodies to a major allergen. *Immunol Lett* 1991; 29: 229-34.
33. VENTAS P, LOMBARDERO M, DUFFORT O, CARREIRA J. Cuantificación de los alérgenos *Der p* I y *Der f* I de los ácaros del polvo doméstico y *Fel d* I de gato mediante ELISA en fase sólida. *Rev Esp Alergol Inmunol Clin* 1990; 5: 71-5.
34. VENTAS P, CARREIRA J, POLO F. Cuantificación del alérgeno mayoritario de *L. destructor* (*Lep d* I) mediante anticuerpos monoclonales. *Rev Esp Alergol Inmunol Clin* 1990; 5 (Suppl. 3): 88.
35. VENTAS P, CARREIRA J, POLO F. Purification and characterization of *Lep d* I, a major allergen from the mite *L. destructor*. *Clin Exp Allergy* 1992; 22: 454.
36. VOORHORST R, SPIEKMA FTHM, VAREKAMP H, LEUPEN MJ, LYKLEMA AW. The house dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces: identity with the house dust allergen. *J Allergy* 1967; 39: 325.
37. WAHN U, SCHWETER C, LIND P, LØWENSTEIN H. Prospective study on immunologic changes induced in two different *Dermatophagoides pteronyssinus* extracts prepared from whole mite culture and mite bodies. *J Allergy Clin Immunol* 1988; 82: 360-70.
38. WENTZ PE, SWANSON MC, REED CE. Variability of cat allergen shedding. *J Allergy Clin Immunol* 1990; 85: 94-8.
39. WOOD RA, EGGLESTON PA, LIND P, et al. Antigenic analysis of household dust samples. *Am Rev Respir Dis* 1988; 137: 358-63.
40. WRAITH DG, CUNNINGTON AM, SEYMOUR WM. The role and allergenic importance of storage mites in house dust and other environments. *Clin Allergy* 1979; 9: 545-61.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.