

The Levels of Cockroach Allergen in Relation to Cockroach Species and Allergic Diseases in Thai Patients

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Cockroach (CR) is a common domestic pest in households around the world especially in tropical zones with a higher density of unsanitary areas.¹ The cockroach has been well established as a vector for viral, bacterial and parasitic infections. Apart from its dirty, repulsive appearance and the fetid odor, the cockroach was first documented as a cause of asthma in hypersensitive patients by Berton and Brown in 1964.² Since then, many subsequent studies have revealed that exposure to the cockroach allergen can cause exacerbation of urban asthma as well as other allergic diseases.³⁻⁸ Nowadays, cockroach allergen is considered to be the second most important indoor allergen after house dust mite allergens.⁹ Asthmatic patients allergic to cockroaches are usually exposed to high levels of cockroach allergen in their homes. Cockroach infestation produces various inhaled allergenic materials, i.e. fragments of whole bodies, fecal and other debris, egg castings and even cast exoskeletons.¹⁰⁻¹²

During the past 15 years, many researchers working on aller-

SUMMARY Recently, cockroaches have been established as the second most important allergen, producing allergic diseases, especially in low socioeconomic populations. In Thailand, about 44-61% of atopic patients were positive to cockroach extract by a skin-prick test. This study examined cockroach allergen levels in relation to cockroach species and allergic diseases in the houses of cockroach-sensitive patients. Sixty households of allergic patients in the Bangkok metropolitan area were surveyed using open- and closed-ended questionnaires. Cockroaches were collected using commercial cockroach traps, while dust samples were obtained from the bedrooms, kitchens and living rooms of the houses using a vacuum cleaner. The cockroaches were counted and their species identified. The levels of cockroach allergens were determined by specific monoclonal antibodies using a monoclonal antibody-polyclonal antibody based sandwich ELISA kit. Six cockroach species were identified: *Periplaneta americana* (American cockroach, 72.15%), *Supella longipalpa* (2.75%, found in only one house), *Periplaneta brunnea* (0.78%), *Periplaneta australasiae* (0.78%), *Neostylopyga rhombifolia* (0.78%), *Blattella germanica* (German cockroach, 0.39%) and nymphs (22.35%). Allergens of the predominant species, *P. americana*, were detectable in all homes studied, with the highest levels in the kitchen areas. The range of allergen levels in house dust varied from 0.40-162.00 µg per g of dust. The median and mean allergen levels in kitchen dust were 59.16 µg and 62.80 µg per g of dust, respectively, while the median allergen level in bedroom dust was only 15.90 µg per g of dust. The German cockroach allergen (*Bla g 2*) was undetectable in any of the houses. In conclusion: *P. americana* was the most common cockroach and may be the species causing allergic diseases, especially asthma, in Thailand, which differs from the USA and Europe

gic diseases in different parts of the world have focused on the cockroach instigated allergy.¹³⁻¹⁶ Central to managing clinical symptoms is reducing the source of the sensitizing allergen and avoidance strategies. Hence, important cockroach allergens have been sequenced, deter-

mined by immunological assays and

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synthetically developed for measuring environmental allergens. *Bla g 1*, *Bla g 2*, *Bla g 4* and *Bla g 5* were identified as *Blattella germanica* allergens whereas *Per a 1*, *Per a 3* and *Per a 7* were determined as *Periplaneta americana* allergens.¹⁷⁻¹⁹ For German cockroach allergen, *Bla g 1*, which is a ~25 kDa protein, pI 3.5 and *Bla g 2* which is a 36 kDa protein, pI 5.2, are more important than the other components. About 40% of cockroach allergic patients have serum IgE specific to *Bla g 1*, which cross-reacted with *Per a 1*.²⁰ *Bla g 2* is more specific and was considered the major allergen of the German cockroach, as 60-80% of CR allergic patients had increased serum IgE specific to *Bla g 2*.¹⁸

In Thailand, it was demonstrated that 44-61% of hypersensitive patients reacted to the cockroach allergen by skin-prick tests.²¹⁻²² National cockroach surveys conducted in 14 provinces of Thailand revealed that the most abundant species of cockroaches was the American cockroach, *P. americana*,²³ similar to the results published by Asahina and Hasegawa²⁴ and Asahina.²⁵ The prevalence of *B. germanica* was only 0.6%, which implied that this species may not be the main problem for cockroach allergy in Thailand, unlike in the USA^{9,26,27} and Europe.²⁸ Despite the high prevalence of cockroaches, epidemiological and ecological data of cockroach species related to cockroach allergy and cockroach allergenic components are still lacking.

Therefore, we report an epidemiological study to demonstrate the relationship between the cockroach species and cockroach allergen levels in homes of cockroach-sensitive patients.

MATERIALS AND METHODS

Patients with the clinical symptoms of allergic diseases including allergic rhinitis, asthma, allergic conjunctivitis and atopic dermatitis were enrolled at the Allergy Clinic of the Department of Pediatrics and Otorhinolaryngology, Faculty of Medicine, Siriraj Hospital. Cockroach hypersensitivity was determined by skin-prick testing using whole body crude antigen of the adult American cockroach. The survey was conducted in sixty patient households in the Bangkok metropolitan area. Open- and closed-ended questionnaires were used to obtain the family history of allergic diseases, dwelling types, household construction, location of the houses, nearby environments, pets, insecticide usage, cockroach sighting in the homes, etc.

The cockroach density was assessed using commercial sticky traps because this trap showed significantly more effective than the other kinds under the laboratory and field evaluation.²³ The trap was a paper device folded into a house-like shape with 4 entrances. Baits and pheromone-attractants for cockroaches were placed in the middle of the sticky area. The traps were placed for 3 days near the walls or at corners in the bedrooms, living rooms and kitchens. The cockroaches caught in each trap were counted and identified as species, using pictorial keys of domestic cockroach species in Thailand and relevant references.

Quantification of cockroach allergen

House-dust samples were obtained from the 60 households using a vacuum cleaner for 5 minutes on a 4-square-meter floor area

of the bedrooms, living rooms and kitchens. The collected dust was mixed, weighed and then sieved through a 0.3 mm mesh screen to obtain fine dust. Extraction of the dust samples was performed by using 0.15 M phosphate buffered saline, pH 7.4 (PBS). One hundred milligrams of dust were mixed with 1 ml of PBS and the preparation was kept at 4°C overnight. The extracts were centrifuged at 12,000 x g at room temperature for 10 minutes and the supernatant stored at -20°C until used.

After dust extraction, cockroach allergen levels were measured using a sandwich ELISA.¹⁸ A mixture of two monoclonal antibodies (MAbs), namely MAb 38G6 and MAb 3C2,²⁹ were used as detection reagents for American cockroach allergens. These two monoclonal antibodies were produced against the crude extract of American cockroach and they showed specificity to the American cockroach antigens and did not cross-react to other heterologous antigens including other cockroach species and inhabitant commensal bacteria such as *E. coli*. The MAb 38G6 bound to the component was between < 207 to 72 kDa of cockroach allergens while MAb 3C2 reaction to the component was between 45 to 40 kDa.²⁹⁻³⁰ The sandwich ELISA was performed as described by Sookrung *et al.*²⁹ The MAb for *Bla g 2* was selected to be used for detection of the German cockroach allergen in this study⁹ since this *Bla g 2* is the major allergen of the German cockroach and this MAb does not react to *Bla g 1* and *Per a 1*, the American cockroach allergens, like *Bla g 1*. The assay is available as a commercial test kit that was developed and manufactured by Indoor Biotechnologies Ltd,

USA.³⁰ The allergen detection test was investigated as described in the protocol.

Data analysis

The percentages of each variable were compared using the Chi-square test. Cockroach allergen levels were quantitative data. Statistical analysis revealed that the data were of abnormal distribution, thus further analysis was carried out using the Kruskal-Wallis analysis, a non-parametric method in the SSPS program. The findings were presented as means, medians and standard deviations. The accepted level of significance was $p < 0.05$. The cockroach prevalence was also calculated by the same method.

RESULTS

The cockroach surveys carried out in the 60 households of the allergic patients revealed that 83% of the houses had experienced cockroach sightings, whereas only 17% had not (Fig. 1). However, cockroaches were trapped in only 56.67% of these dwellings. On average, 61.6% of cockroaches were caught in the kitchens, while the percentage of cockroaches caught in the living rooms, bedrooms and single room apartments were 26.72%, 8.19% and 3.44%, respectively. The numbers of cockroaches trapped in the kitchens were significantly higher ($p < 0.05$) than those obtained in other places (Fig. 2).

All affected patients or their guardians were advised about intervention modalities. The percentage of those using insecticides was 75, whereas 25% refrained from using insecticides or only using them occasionally (less than 5 times a year).

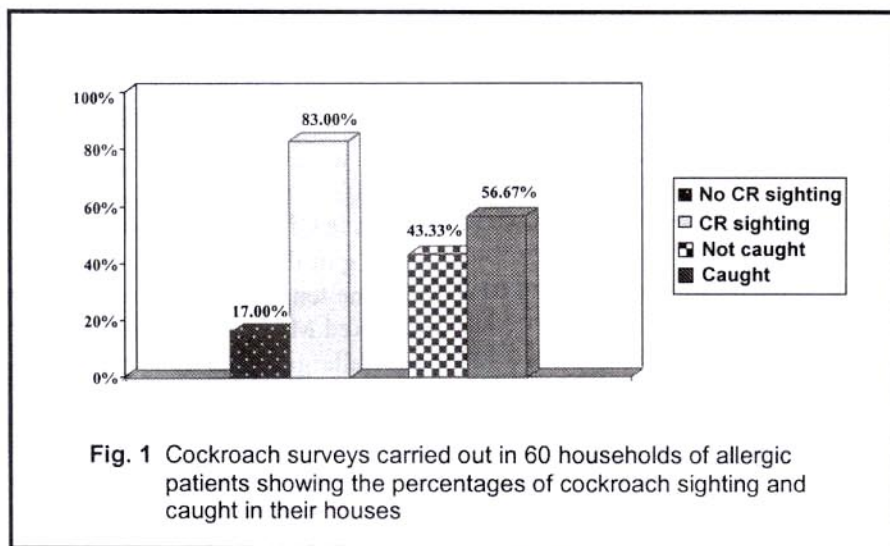


Fig. 1 Cockroach surveys carried out in 60 households of allergic patients showing the percentages of cockroach sighting and caught in their houses

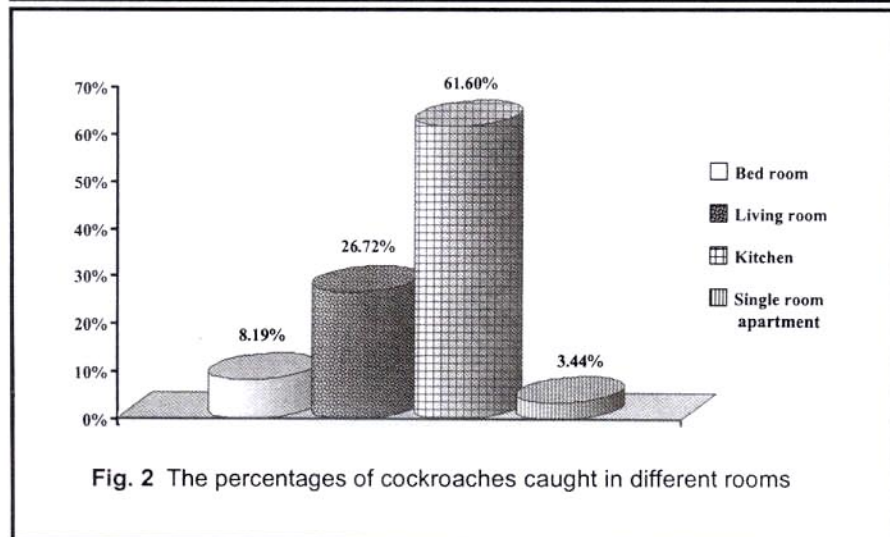


Fig. 2 The percentages of cockroaches caught in different rooms

The insecticides were mainly used in value. The levels of cockroach allergen in house dust were 0.04-162 µg per g of dust in bedroom dust samples, 0.06-152 µg per g of dust in living room dust samples and 0.20-194 µg per g of dust in kitchen dust samples. The highest mean and median allergen levels were 59.16 µg and 62.80 µg per g of dust, respectively, in kitchen dust samples, using MAb3C2. The median allergen level determined by the mixed MAb from bedrooms (5.6 µg per g of dust) and living room dust samples (24.00 µg per g of dust) were significantly different from those determined by 38G6 and 3C2 MAbs. Although the

spray form (94.88%) (Fig. 3). Six species of cockroaches and nymphs were caught: *Periplaneta americana* (72.15%), *Supella longipalpa* (2.75%), *Periplaneta australasiae* (0.78%), *Periplaneta brunnea* (0.78%), *Neostylopyga rhombifolia* (0.78%), *Blattella germanica* (German cockroach, 0.39%) and nymphs (22.37%) (Fig. 4).

The distribution of allergen levels in the houses is reported in Table 1, which indicates significant differences in the same statistical

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median allergen levels determined by the mixed MAb and MAb38G6 from kitchen dust samples were not significantly different, these median levels were significantly different from the median level determined by MAb3C2, which gave the highest levels. Regarding allergen distribution in the homes, the mean and median allergen levels of bedrooms, living rooms and kitchens detected by the same MAb were compared. The mean allergen level of bedroom dust using mixed MAbs was 12.78 μ g per g of dust, which was 2-fold lower than the mean allergen level from living rooms (25.74 μ g per g of dust) and nearly 3-fold lower than that of kitchens (35.42 μ g per g of dust). Statistical analysis revealed that there was no significant difference between the results from living rooms and kitchens but these mean levels were significantly different from that of the bedrooms. The mean and median allergen levels obtained from MAb3C2 gave the highest yield when compared to the other MAbs.

However, the median allergen level of the living rooms (46.00 μ g per g of dust) was not significantly different from that of the kitchen dust (62.80 μ g per g of dust), but they were significantly different from the median level of the bedrooms (15.90 μ g per g of dust). This result showed the same tendency as the result from the mixed MAbs. The German cockroach allergen (*Bla g 2*) was undetectable in any of the houses.

DISCUSSION

In cockroach surveys of the households of 60 cockroach-sensitive patients located in Bangkok (Fig. 1), 83% reported cockroach sighting in their homes, while a study from inner-city New York, USA, reported only 67%.³⁰ The result indicates that Bangkok may have a higher density of cockroaches. The prevalence of cockroaches in the surveyed houses

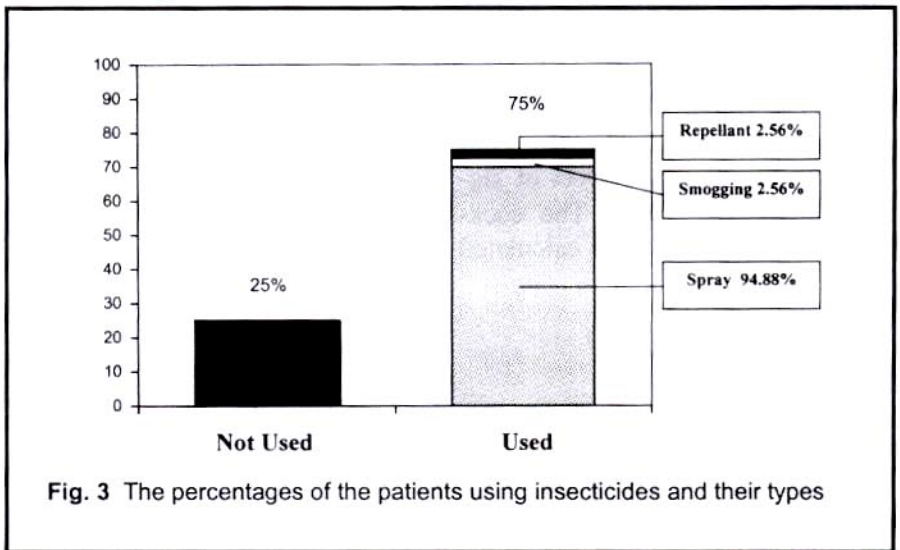


Fig. 3 The percentages of the patients using insecticides and their types

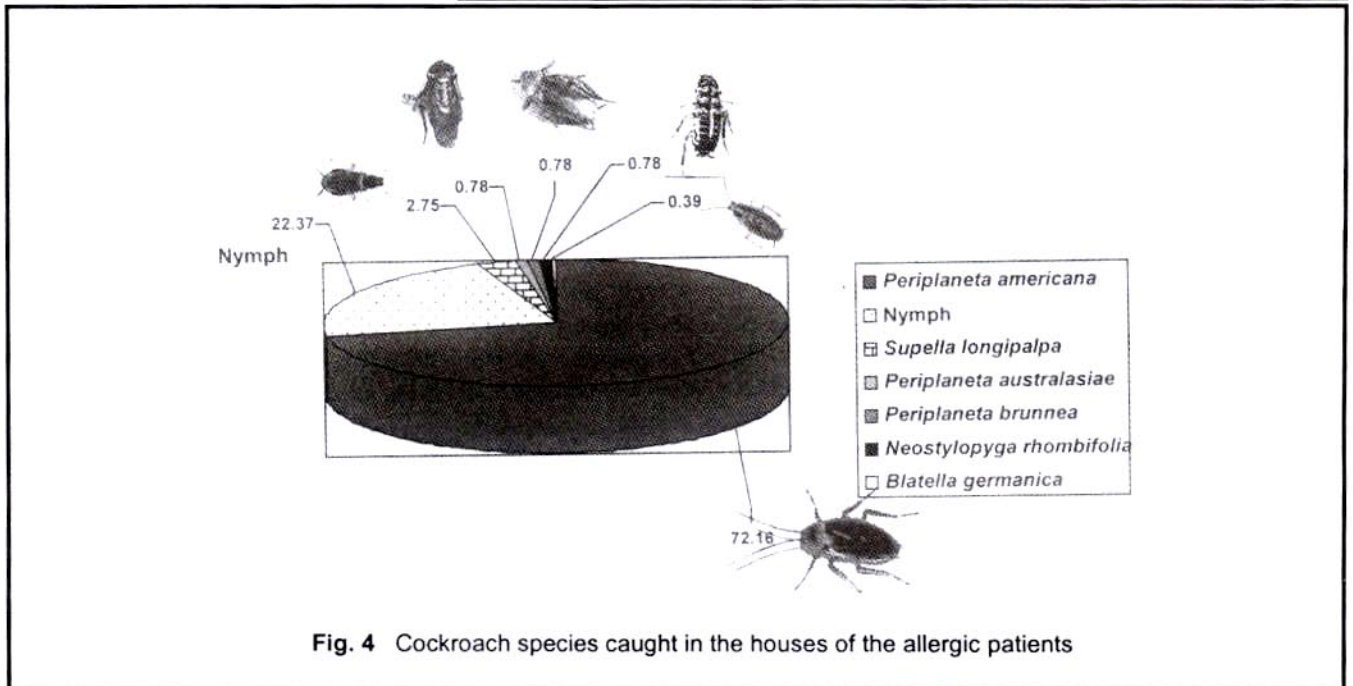


Fig. 4 Cockroach species caught in the houses of the allergic patients

Table 1 Statistical inferences of different test methods in specific places and comparison using multiple comparisons

Statistical value	Bedroom			Living room			Kitchen		
	Mixed	38G6	3C2	Mixed	38G6	3C2	Mixed	38G6	3C2
Mean ($\mu\text{g/g}$ of dust)	12.78 ^a	21.23 ^b	44.34 ^b	25.74 ^a	40.22 ^b	57.47 ^b	35.42 ^a	41.05 ^a	59.16 ^b
Median	5.6 ^a	11.40 ^b	15.90 ^b	24.00 ^a	40.10 ^b	46.00 ^b	28.00 ^a	51.20 ^a	62.80 ^b
S.D.	13.71	21.53	47.05	21.53	26.14	51.44	38.49	25.45	54.88
Minimum	0.04	0.96	0.40	0.08	5.00	0.06	0.20	4.40	0.40
Maximum	48.00	80.40	162.00	83.60	90.40	152.00	152.80	82.00	194.00

^{ab}Any difference in index letters along the same horizontal line indicates a difference of $p < 0.05$ using Kruskal-Wallis analysis of variance and multiple comparisons

was 56.67%, which was much lower than the reported cockroach appearances. However, this prevalence was similar to the results of a previous survey of cockroaches in Thailand.²³ Reasons for the difference in reported cockroach sighting and the prevalence of trapped cockroaches might be insecticide usage, cockroach habits and their major habitat. The affected patients were advised to reduce indoor allergens in their homes. Between the various modalities, insecticides are the most common and easiest method, which is reflected in the percentage of their usage (75%).

Our findings were similar to previous studies. The most abundant cockroach species was *Periplaneta americana* (72.15%) while only a small percentage of *Blattella germanica* (German cockroach) was found (0.39%) (Fig. 4). The *B. germanica* density in this study was nearly equal to a cockroach survey conducted in 14 provinces of Thailand. However, the result was much different from the prevalence of *B. germanica* reported in a study in 4 hospitals (8.1%).³² The hospital environment is different from that of a household. The traps were also placed in the food centers and stock rooms

in the hospitals which were major habitats of *B. germanica*. In our study, *P. fuliginosa*, which is the main species causing allergic diseases in Japan^{33,34} and *B. orientalis*, which is associated with asthma in Spain,²⁸ were not caught, differing from the data of the national cockroach survey in Thailand which reported a density of *P. fuliginosa* at 0.5%.

A commercial diagnostic test kit for measuring American cockroach allergen levels in house dust samples is not yet available. Fortunately, Sookrung *et al.*²⁹ have successfully produced two specific monoclonal antibodies (MAB38G6 [< 207 to 72 kDa] and 3C2 [45 to 40 kDa]) to *P. americana* allergenic components. The epitope of Mab3C2 was found to be *Per a* 1.0105.³⁰ A sandwich ELISA using the mixture of these 2 specific MAbs as capture antibody has been developed for the quantitative measurement of cockroach allergen loads in dust samples. These 2 MAbs were mixed in order to gain a higher sensitivity. In this study, these 2 specific MAbs and their mixture (mixed MAbs) were used to study the distribution of cockroach allergen levels in the houses. The results revealed that the

mixed MAbs gave a lower sensitivity with lower amounts of allergen under the same conditions. Comparing the two specific MAbs, the data indicated that MAB3C2 had a higher yield, though not statistically different from MAB38G6. The outcome of the raw data showed that the mixing process caused interference rather than synergy.

B. germanica allergens in the house dust were measured using a commercial test kit. Specific MAB for *Bla g* 2 was used, as it is the major allergen of *B. germanica*. Our results showed that American cockroach allergen was detectable in all houses studied, but *B. germanica* allergen was not detected in any house, corresponding to the very low prevalence of *B. germanica* (0.39%) in the patients' households. There may have been German cockroach allergen, but at too low levels to be detected, which suggests that in Thailand *B. germanica* is a less important cause of allergic diseases than *P. americana*. A similar result was reported from Durban, South Africa which is also located in the tropical zone.³⁵ A study in Japan reported *P. fuliginosa*, which is in the same genus as *P. americana*, as the

major cockroach species.³³ The observations of the studies mentioned imply that in Asia and the tropical zones the *Periplaneta* group is more related to allergic diseases, whereas in the USA and Europe it is the *Blattella* group.

In contrast to house dust mites whose main site of exposure is the bedroom, the highest density of cockroaches was found in the kitchens (61.6%), similar to all previous studies, since there is most food debris, suggesting that the kitchen should be the main site of exposure. The living room is where family members spend time together, watching TV usually with some snacks, coffee and drinks, also leaving food debris. Thus, the living room was the second most common place for trapping cockroaches after the kitchen (26.72%). Pollart *et al.*³⁶ reported that kitchen dust contained 50-fold more cockroach allergens than bedroom dust. However, we found that the mean level of the cockroach allergen in kitchens and living rooms obtained only 2- to 3-fold more than the bedrooms while the median level was 4- to 5-fold higher. Considering the location in a tropical country, the house conditions, prevalence of cockroach sightings, and sanitation of the patients' houses, our result seems to reflect the different living conditions.

The sensitizing level of house dust mite was established at 2 µg per g of dust. Studies in the USA showed the best correlation between sensitization and allergen amount in the bedroom, even though the highest levels were in the kitchen.^{9,19} The studies reported that the threshold level of cockroach allergen exposure for sensitization was 2 units per g of allergen, whereas 8 units per g of allergen caused asthma symptoms.¹⁹ The longer

exposure time and high allergen levels from this study may lead us to reconsider the bedroom as the main site of exposure. An environmental assay for the cockroach allergen in the dust from Tampa, South Florida, reported levels from 2-200,000 ng of *Per a 1* eq/g.¹⁷ Most samples (83%) contained 10-10,000 ng of *Per a 1* eq/g.¹⁹ Dust samples from the homes of patients and the Allergy Clinic at the Children's Mercy Hospital were examined for the American cockroach allergen. The concentrations varied from 2.5-26.5 µg per g of the cockroach allergen.³⁷

Our results cannot be directly compared to the previous reports, or even the threshold level of German cockroach allergen, since they show the data in different units. Therefore, further studies should be done on allergen levels in houses of the normal population to determine cockroach allergens in the environment, the sensitizing levels and thresholds for asthmatic attacks, and the main sites of exposure.

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