

Exposure Assessment of Indoor Allergens, Endotoxin, and Airborne Fungi for Homes in Southern Taiwan

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This study was undertaken to examine the seasonal variations of domestic *Der p 1*, *Der p 2*, and endotoxin on mattress and airborne fungal concentrations in homes of asthmatic and nonasthmatic children in southern Taiwan, where temperature and relative humidity are usually high throughout the year. A group of asthmatic children (10–12 years old) were selected randomly based on a citywide questionnaire survey. The nonasthmatic children were chosen to be in the comparison group by matching in age, gender, and proximity of residence. Environmental sampling of domestic microbes was conducted once a month for a year. Twelve calendar months were grouped into spring, summer, fall, and winter according to weather data (mainly average temperature and humidity) from the Central Weather Bureau. Dust samples from a child's mattress and airborne samples from a child's bedroom were collected and analyzed for allergens of *Der p 1* and *Der p 2*, endotoxin, and fungi respectively. Results show that about 65% of children's mattresses in our region have *Der p 1* levels greater than 2 µg/g. It is also apparent that most airborne fungal concentrations found in homes of either asthmatic or nonasthmatic children are higher than the recommended levels of concern. The predominant genera are *Cladosporium*, *Aspergillus*, *Penicillium*, *Alternaria*, and yeast. In addition, seasonal effects seem to be a critical factor for the concentrations and distributions of domestic endotoxin in these study homes. The implication of long-term exposure to these high levels of environmental microbes and how their effects vary with seasons remain to be further characterized. © 2001 Academic Press

Key Words: airborne fungi; allergen; endotoxin; *Der p 1*, *Der p 2*.

INTRODUCTION

Asthma is a multifactorial disease that has been attributed partly to genetic predisposition and triggered by chemical irritants, virus infection, or environmental allergens (Meyers *et al.*, 1994; Sandford *et al.*, 1996; Infante-Rivard, 1993; Dekker *et al.*, 1991; Korpp, 1988). Seroepidemiological studies of large populations have indicated a dominant correlation between serum titers of immunoglobulin E (IgE) and reported asthma (Call *et al.*, 1992; Charpin *et al.*, 1991; Gelber *et al.*, 1993). The observation supports allergy as a major determinant of asthmatic phenotype. The primary event in allergy is an immune response to a normally innocuous foreign protein, an allergen, and most allergens associated with allergy are predominantly found indoors. It becomes essential to characterize the levels of allergen exposure in developing intervention strategies for childhood asthma.

The well-known indoor allergens associated with allergic diseases are house dust mite, dander from pets, cockroach, and fungal allergens (Luczynska *et al.*, 1990; Swanson *et al.*, 1985; Pollart *et al.*, 1991). Among them, aeroallergens derived from the house dust mite (*Dermatophyoides pteronyssinus*) are powerful inducers of allergic responses in children (Tsai *et al.*, 1988; Tang *et al.*, 1986; Dowse *et al.*, 1985; Sporik *et al.*, 1990). Dust mite is also the primary source of antigen in house dust, with the principle component being a proteolytic enzyme (*Der p 1*), 95% of which were found in mite feces (Tovey *et al.*, 1981). *Der p 2* is an antigen that belongs to the group II allergens and is associated with mite bodies (Chapman, 1989). Studies have showed that more

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than 80% of asthmatic children are sensitized to these proteins (Sarsfield, 1974; Smith *et al.*, 1969, Pollart *et al.*, 1989). Several studies have also demonstrated the relationship between levels of *Der p 1* allergen in mattresses and allergic respiratory symptoms, such as onset of wheezing (Sporik *et al.*, 1990; Lau *et al.*, 1989). Although the evidence for a dose-response effect between mite allergen exposure and allergic disease severity has been indirect and largely circumstantial, the importance of exposure to mite allergen has been well proved as major risk factor in the development of allergic disease, asthma in particular (Platts-Mills and De Weck, 1989; Platts-Mills *et al.*, 1992).

Fungi are ubiquitous and could utilize many different substances for growth. Most fungi that grow in domestic environments are often accompanied by domestic dampness (Hunter *et al.*, 1988; Veroeff *et al.*, 1992). However, the prevalence of airborne fungi is highly variable and determined by many factors, such as climatic factors, agriculture environments, and some specific reservoirs of contamination. Fungi are a well-known allergen source and many fungal spores contain allergens causing one or several allergic diseases. Serial studies have reported that children living in damp and moldy dwellings show a greater prevalence of respiratory symptoms (cough, phlegm, wheeze, sore throat, and runny nose) and asthma (Hu *et al.*, 1997; Cuijpers *et al.*, 1995). While the health implications of living in damp environments is of significant concern, the effect of airborne fungal exposure on the development and severity of asthma has not been conclusively established, and the mechanism of fungus-induced asthmatic reactions is still mostly unclear. Kauffman has observed that sensitization to fungi is age-dependent and declines rapidly with age (Kauffman *et al.*, 1995). Therefore, sensitization to fungal allergens can be a serious threat for patients with asthma and atopic symptoms and may be a risk factor for respiratory illness in children and young adult.

Endotoxins are partly formed from the outer cell membrane of gram-negative bacteria. Several experimental studies have shown that they are potent, proinflammatory substances present in domestic and some occupational environments (Schwartz *et al.*, 1995; Smid *et al.*, 1992). It has been suggested that chronic exposure to inhaled endotoxin might be associated with the development and severity of asthma (Michel *et al.*, 1996). A cross-sectional study, conducted by Michel and colleagues, investigated the content of house dust mite (HDM) allergen and endotoxin. They concluded that for HDM-sensitized

subjects with exposure to a high level of allergen, the endotoxin measured in HD is an important determinant of asthma severity (Michel *et al.*, 1996). Taiwan is located in a subtropical region with high temperatures and relative humidity throughout the year, an optimal condition to sustain these bioaerosols and aeroallergens indoors. Our previous cross-sectional investigations showed that the exposure levels of domestic dust mite allergens, airborne fungi, and endotoxin appear to be an important concern for domestic environments (unpublished data). The aim of this study was therefore to examine the seasonal variations of domestic *Der p 1*, *Der p 2*, and endotoxin in mattress and airborne fungal concentrations in homes asthmatics and nonasthmatics.

MATERIALS AND METHODS

Study Sites

A group of asthmatic children (10–12 years old) were selected based on a citywide random survey conducted prior to the environmental sampling activity. A selected variable in the questionnaire asked the parents to report whether the child had been diagnosed previously by a physician as “asthmatic.” That was how the asthmatic children were selected in the first phase. Furthermore, the clinical class of these recruited subjects was reexamined by a pediatrician at the National Cheng Kung University Hospital before they were included into the year-long study. The healthy children, free of allergic symptoms, were chosen to be the comparison group by matching their age, gender, and proximity of residence. The homes of these children became the study sites, distributed throughout the city. Sampling was conducted every month for a year. Twelve calendar months were grouped into spring (March, April, and May), summer (June, July, August, and September), fall (October and November), and winter (December, January, and February) according to the weather data (average temperature and humidity) from the Central Weather Bureau of southern Taiwan. Twenty-three homes of asthmatic children and 12 nonasthmatic completed all 12-month measurements.

Environmental Measurements

Der p 1 and Der p 2 from dust samples. Dust samples from a child's mattress were collected by a hand-held vacuum cleaner (National MC 33000) with a glass fiber filter. Samples were collected by

vacuuming a 1-m² area for 3 min. After the sampling, the samples were sealed with parafilm and stored at 4°C before handling. Dust samples were pre-screened with a sterile mesh screen (300 µm) to remove the large particles and hairs and then weighed. All dusts were separated into two tubes, one for allergen analysis and one for endotoxin analysis. After weighting, all samples were stored with desiccating agents until extraction. Samples were held in BBS-T (0.1% Tween 20 in borate buffer saline, pH 8.0) and extracted in the ultrasonic vibrator for 2 h at 4°C. *Der p 1* and *Der p 2* were measured by two-sided, monoclonal antibody-based ELISA (enzyme-linked immunosorbant assay) (Platts-Mills and Chapman, 1987). The extract standard and monoclonal antibodies were purchased from the Lymphocyte Culture Center (Indoor Biotechnologies Inc.). The allergen concentrations were expressed in micrograms of measured allergen per gram of dust.

Endotoxin from dust samples. Dust samples for endotoxin analysis were collected concurrently with those for allergen analysis. After screening and weighing, samples were transferred into glass tubes. Desiccation units were attached to preserve each sample after collection, at 4°C, until extraction. Samples were extracted by 0.05 M potassium phosphate, 0.01% triethylamine, pH 7.5, buffer bath sanitation for 60 min at 20°C and analyzed by the kinetic Limulus assay with resistant-parallel-line estimation (KLARE) (Milton *et al.*, 1990; Walters *et al.*, 1994).

Airborne fungi. We measured the airborne fungal concentration in a child's bedroom and outside entrance of the home. Duplicate samples of airborne fungi were collected using a Burkard sampler, a single-stage hole-to-agar impactor with 100 holes (Model PASA/B, Burkard Mfg. Co., Rickmansworth, UK) with malt extract agar plates (MEA) at a flow rate of 10 lpm (Macher *et al.*, 1995). The sampling height was 1.2 to 1.5 m near the human breathing zone. Between the measurements, the samplers were swabbed with 70% ethanol. After field sampling, the plates were incubated at 25°C for 5 days. Fungi were identified morphologically according to mycology references (Larone, 1987; Barnett and Hunter, 1987). The reported genera included at least *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Paecilomyces*, *Curvularia*, *Fusarium*, *Trichoderma*, the nonsporing fungi, and yeast. The yields of colonies were then corrected by using a positive hole

conversion table, constructed based on a statistical distribution model, before the calculation of final concentrations, colony forming unit per cubic meter (CFU/m³).

Climatic condition. The temperature and relative humidity were documented using a continuous recorder (StowAway) in these sampling locations. The data logger was connected to computer to download all readings of temperature and relative humidity, and the time interval between each reading could be as short as 4.8 min in the study week.

Statistical Methods

The EXCEL package was used for data entry and management and SATVIEW for statistical analysis (Abacus Concept, Inc). A nonparametric statistical test was used in examining the differences in averaged concentrations between seasons and homes of asthmatics and nonasthmatics.

RESULTS

A total of 23 asthmatics and 12 nonasthmatics completed all environmental measurements of interest, namely, the levels of airborne fungi, dust allergens, and endotoxin.

Temperature and Relative Humidity

The ranges of temperature and relative humidity documented in children's homes were 22.5–29°C and 60–78% throughout the year. There was no significant difference, with respect to these two climatic factors, between homes of asthmatics and nonasthmatics.

Dust Mite Allergens (*Der p 1* and *Der p 2*)

There was no correlation between variation of relative humidity and *Der p 1*/*Der p 2* concentrations. In spring, 67.4% of the sampling houses showed *Der p 1* concentrations higher than the environmental recommended levels, 2 µg/g (Lau *et al.*, 1989; Platts-Mills and De Weck, 1989). The percentages of houses with concentrations exceeding the above level were 64.3% in summer, 63.6% in fall, and 77.8% in winter. Table 1 shows the seasonal *Der p 1* concentrations in homes of asthmatics and nonasthmatics. The highest concentration occurred in winter (10.15 µg/g), followed by 9.11 µg/g in fall, and the lowest level was found in spring (3.78 µg/g).

TABLE 1

The Seasonal *Der p 1* Concentrations ($\mu\text{g/g}$ Dust) in Homes of 23 Asthmatic and 12 Nonasthmatic Children

	Spring	Summer	Fall	Winter	<i>P</i> value ^a
Total	3.78	5.61	9.11	10.15	0.0508*
Asthmatics	4.08	3.76	9.19	11.75	0.0696
Nonasthmatics	3.24	8.99	8.96	7.10	0.2581
<i>P</i> value ^b	0.6266	0.2205	0.6767	0.5316	

^aNonparametric Kruskal–Wallis test comparing the concentrations between four seasons.

^bNonparametric Mann–Whitney test comparing the concentrations between homes of asthmatic and nonasthmatic children.

* $P < 0.05$.

The levels at asthmatic homes were generally higher than those at nonasthmatic homes, but without significant difference. Table 2 shows the *Der p 2* concentrations in four seasons inside asthmatic and nonasthmatic homes. Except for winter, the concentration variation of *Der p 2* and its difference between asthmatics and nonasthmatics present a very similar pattern, as *Der p 1* has shown (Table 1). The highest level of *Der p 2* occurs in fall for asthmatic homes and in winter for nonasthmatic. There is a good correlation between *Der p 1* and *Der p 2* concentrations in all samples analyzed ($r = 0.566$, $P < 0.01$).

Endotoxin

Our previous pilot study found that endotoxin levels measured from 8-h airborne samples in domestic environments were generally lower than the LAL detection limit (75%) (unpublished data). Therefore, endotoxins were extracted and analyzed from only the dust samples in this investigation.

TABLE 2

The Seasonal *Der p 2* Concentrations ($\mu\text{g/g}$ Dust) in Homes of 23 Asthmatic and 12 Non-asthmatic Children

	Spring	Summer	Fall	Winter	<i>P</i> value ^a
Total	1.41	1.49	1.96	3.32	0.0321*
Asthmatics	1.51	0.98	2.57	2.00	0.0339*
Nonasthmatics	1.21	2.42	0.79	5.83	0.1087
<i>P</i> value ^b	0.2661	0.0255*	0.7545	0.8894	

^aNonparametric Kruskal–Wallis test comparing the concentrations between four seasons.

^bNonparametric Mann–Whitney test comparing the concentrations between asthmatic and nonasthmatic homes.

* $P < 0.05$.

TABLE 3

The Seasonal Endotoxin Concentrations (ng/mg Dust) in Homes of 23 Asthmatic and 12 Nonasthmatic Children

	Spring	Summer	Fall	Winter	<i>P</i> value ^a
Total	45.18	17.96	50.25	18.84	0.0002*
Asthmatics	51.47	17.16	62.23	19.47	0.0010*
Nonasthmatics	33.13	19.98	28.31	17.59	0.2302
<i>P</i> value ^b	0.6266	0.2205	0.6767	0.5316	

^aNonparametric Kruskal–Wallis test comparing the concentrations between four seasons.

^bNonparametric Mann–Whitney test comparing the concentrations between asthmatic and nonasthmatic homes.

* $P < 0.05$.

Table 3 shows the seasonal endotoxin concentrations (ng/mg dust) in asthmatic and nonasthmatic homes. In total sampling houses, two peak concentrations were observed in spring and fall (45.18 and 50.25 ng/mg dust), which are twofold the levels observed in summer and winter (17.96 and 18.84 ng/mg dust). However, the levels in spring and fall in asthmatic homes are generally higher than those in nonasthmatic homes.

Airborne Fungi

Table 4 shows the seasonal indoor fungal concentrations in asthmatic and nonasthmatic homes. The average total fungal concentrations were generally high and the highest level occurred in winter (20,551.7 CFU/m³), with a significant difference ($P < 0.0001$) compared to the other seasons. The most predominant indoor genera were *Cladosporium*, *Aspergillus*, *Penicillium*, and *Alternaria*, regardless of season. *Cladosporium* ranked first with about 85% of identified colonies from all indoor samples, giving the highest concentration in winter, 17,457.1 CFU/m³, with a significant difference when compared to other seasons. The lowest colony counts of *Cladosporium* were obtained during summer (2160 CFU/m³). In summer, *Aspergillus* showed the highest concentration (631.4 CFU/m³) in indoor environments across seasons. *Penicillium* was another genus consistently distributed indoors and outdoors in every season. *Alternaria* showed a significantly high level in spring (3402.4 CFU/m³), with generally low concentrations in other seasons.

The seasonal variation in fungal distribution is similar in asthmatic and nonasthmatic homes. The fungal exposure variations between asthmatic and non-asthmatic homes appear, however, to be inconsistent.

TABLE 4
Concentrations of Major Fungi (CFU/m³) in Asthmatic and Nonasthmatic Homes over 4 Seasons

	Spring	Summer	Fall	Winter	P-value ^a
Total samples (n = 35)					
Total CFU	11096.4	6798.1	11083.1	20551.7	< 0.0001
<i>Aspergillus</i> spp.	279.3	631.4	291.7	353.1	0.1780
<i>Penicillium</i> spp.	760.3	462.9	462.7	421.2	0.5324
<i>Cladosporium</i> spp.	4607.2	2160.0	6557.5	17457.1	< 0.0001
<i>Alternaria</i> spp.	3402.4	32.7	119.0	280.3	< 0.0001
Yeast	684.2	459.5	1660.7	594.2	0.0007
Asthmatics (n = 23)					
Total CFU	11233.0	7288.9	10727.3	20676.1	< 0.0001
<i>Aspergillus</i> spp.	306.7	738.0	303.1	451.2	0.0909
<i>Penicillium</i> spp.	839.6	568.4	454.0	496.8^b	0.2727
<i>Cladosporium</i> spp.	4972.9	2085.0	6469.5	17696.0	< 0.0001
<i>Alternaria</i> spp.	3039.1	47.4	87.9	251.0	< 0.0001
Yeast	670.8	627.8	1529.3	594.1	0.1137
Nonasthmatics (n = 12)					
Total CFU	10834.4	5857.5	11765.2	20313.3	0.0003
<i>Aspergillus</i> spp.	226.9	427.0	269.8	165.0	0.6543
<i>Penicillium</i> spp.	608.3	260.7	479.3	276.3	0.2999
<i>Cladosporium</i> spp.	3906.1	2303.9	6726.1	16999.3	< 0.0001
<i>Alternaria</i> spp.	4098.6	4.5	178.8	336.5	< 0.0001
Yeast	709.8	136.5	1912.5	594.3	0.0054

^aNonparametric Kruskal-Wallis test comparing the concentrations between four seasons.

^bSignificant difference in comparing the concentrations between asthmatic and nonasthmatic houses using nonparametric Mann-Whitney test ($P < 0.05$).

To identify the potential fungal reservoir, indoor/outdoor comparisons were used to document the presence of biologically derived contamination. The indoor/outdoor concentration ratios (I/O ratio) were calculated at every house for each season (Table 5). The I/O ratios of *Cladosporium*, *Aspergillus*, *Penicillium*, *Alternaria*, and yeast were often lower than 1. More than 60% of investigated houses

gave an I/O ratio of *Cladosporium* lower than 1 in every season. Similarly, more than 62.9% of homes gave an I/O ratio of *Alternaria* lower than 1 in spring and winter.

The Pearson correlation was used to examine the relationship between indoor and outdoor concentrations of fungal genera during four different seasons (Table 6). *Cladosporium* is the only genus with a significant correlation between indoor and outdoor concentrations at all seasons, and the correlation coefficient was 0.571 ($P < 0.01$) in spring, 0.403 ($P < 0.05$) in summer, 0.668 ($P < 0.01$) in fall, and 0.715 ($P < 0.01$) in winter respectively. The indoor *Aspergillus*, *Penicillium*, and *Alternaria* were also significantly correlated with outdoor concentrations, but only in spring, $r = 0.700$, 0.451, and 0.405, respectively.

DISCUSSION

Dust Mite Allergens (*Der p1* and *Der p2*)

The year-round warm and humid climatic condition in Taiwan could have provided an optimal environment for the growth of *D. pteronyssinus*. That may be why we observe a generally high level of *Der p 1* concentrations throughout the year in our region. Epidemiological studies from the United States, Australia, Germany, and England have shown that exposure to more than a threshold level of 2 µg/g group I mite allergen (*Der p 1*) will increase the risk of sensitization to mites and that exposure to ≥ 10 µg/g will increase the risk for inducing asthmatic symptoms (Sporik *et al.*, 1990; Charpin *et al.*, 1991; Peat *et al.*, 1987; Arruda *et al.*, 1991). Our results indicate that about 65% of children's mattresses in our region had levels higher than 2 µg/g, and the concentrations seemed to be similar to what has been found in northern Taiwan before (Li and Hsu, 1997). How this high percentage of con-

TABLE 5
Percentage of Houses with Indoor to Outdoor (I/O) Ratios Smaller than 1, between 1 and 2, and Greater than 2

Percentage of houses with I/O ratio at various levels ^a	Spring			Summer			Fall			Winter		
	≤ 1	$>1, \leq 2$	>2	≤ 1	$>1, \leq 2$	>2	≤ 1	$>1, \leq 2$	>2	≤ 1	$>1, \leq 2$	>2
<i>Aspergillus</i>	34.29	25.71	5.71	31.43	14.29	20.00	31.43	20.00	17.14	37.14	11.43	14.29
<i>Penicillium</i>	34.29	11.43	20.00	45.71	14.29	17.14	54.29	14.29	17.14	51.43	20.00	8.57
<i>Cladosporium</i>	60.00	31.43	5.71	62.86	20.00	8.57	68.57	31.43	0	68.57	17.14	2.86
<i>Alternaria</i>	68.57	5.71	11.43	22.86	2.86	0	22.85	5.71	2.86	62.86	5.71	11.43
Yeast	45.71	14.29	14.29	42.86	5.71	8.57	34.29	2.86	37.14	40.00	14.29	14.29

^aIndoor/outdoor ratio; the samples with outdoor concentrations of 0 are not included.

TABLE 6

The Relationship between Predominant Genera Found Indoors and Outdoors (using Pearson Correlation Examination)

Correlation coefficient	Spring	Summer	Fall	Winter
<i>Cladosporium</i>	0.571**	0.403*	0.668**	0.715**
<i>Aspergillus</i>	0.700**	0.082	-0.018	-0.103
<i>Penicillium</i>	0.451*	0.365	0.028	0.289
<i>Alternaria</i>	0.405*	NA	0.613	0.306
Yeast	0.009	0.399	0.139	0.283

Note. The concentrations were natural logarithm transformed in Pearson's correlation examination.

** $P < 0.01$.

* $P < 0.05$.

taminated homes is associated with the increasing prevalence of children with asthma in this country (Hsieh and Shen, 1988; Li and Hsu, 1997; Su *et al.*, 1998) deserves further research.

Serial studies have supported that mite densities exhibit a seasonal cycle that coincides with relative humidity (RH) changes, with the highest mite concentrations occurring during periods of high RH (Charlet *et al.*, 1978; Arlian *et al.*, 1982; Colloff, 1991). This phenomenon has not been observed in our region, where high RH and temperature are present most of the time. However, the pattern of increasing concentrations from spring to winter may be in part explained by traditional housekeeping practices. The thorough home cleaning is likely to be performed at the end of the year in Taiwan for preparation of the Chinese New Year festival and results in a lower concentrations observed in spring.

Our results suggest that the levels of *Der p 1* and *Der p 2* in asthmatic homes are in general higher than in nonasthmatic homes throughout the year. The finding is consistent with studies conducted in Stockholm and England, showing that the *Der p 1* concentrations on mattress surfaces are higher in the homes of atopic children than those of non-atopic children (Nordvall *et al.*, 1988; Wickman *et al.*, 1991). Meanwhile, we are confident that our 1-year-long sampling could well represent the average exposure levels and patterns of homes in southern Taiwan because of the random selection of homes and the comparison of their housing characteristics with those of homes from our background survey. Further studies are needed to verify the public health implications for children in Taiwan, whose environments generally have such high levels of house dust mite allergens.

Endotoxin

Michel *et al.* measured endotoxin from dust samples that were collected from mattresses in two studies and the average concentrations were 0.518 and 1.78 ng/mg dust. Horak *et al.*, also measured the endotoxin levels in mattresses of six asthmatics and four nonasthmatics (40.6 and 223.5 ng/mg dust). The results appear to suggest that endotoxin levels are higher in nonasthmatic homes. The average concentrations documented in our region were higher than the levels found in Belgium (Michel *et al.*, 1991), and the concentrations measured in spring and fall at asthmatic homes were higher than those in Poland (Hork *et al.*, 1996).

"Season" seems to be a determinant factor for domestic endotoxin levels in these investigated houses. In addition, the environmental endotoxin measurements from different laboratories are not necessary comparable because the LAL reagents themselves vary widely in their sensitivity to environmental endotoxins (Milton *et al.*, 1990; Walters *et al.*, 1994). Because of that, it has been difficult to establish a TLV (threshold limit value) for endotoxin exposure. The American Conference of Governmental Industrial Hygienists (ACGIH) proposed a practical alternative approach for limiting endotoxin exposure using a relative limit value (RLV) (ACGIH, 1999). This approach is based on comparison of endotoxin activity levels in the environment in question with simultaneously determined background levels. As a result, 10 times background is proposed as an RLV action level in the presence of respiratory symptoms and 30 times background is a maximum RLV in the absence of symptoms. Therefore, to collect appropriate background endotoxin measurements in our region may be the key to determining the level of endotoxin exposure and the degree of its health effects on our population.

Although the role of endotoxin in the development of asthma is still unclear, most studies support the theory that the adjuvant properties of endotoxin may contribute to allergic sensitization (Michel *et al.*, 1996, 1992). Future investigations should aim to clarify how that particular property affects the mechanism of the development of asthma.

Airborne Fungi

The relationships between airborne fungi and adverse health effects, such as fungus-induced asthmatic reactions, are not well understood. Epidemiological studies of dose-response relationships are also not completely established. Therefore,

there are no TLVs at the time for fungal exposure in indoor environments. However, some numerical recommendations have been published for controlling indoor microbiological contamination (ACGIH, 1986, 1989; AIHA, 1996). It is obvious that the domestic fungal concentrations in our region are much higher than these recommended levels in both asthmatic and nonasthmatic homes. Most fungi produce highly allergic proteins or glycoproteins that could cause hypersensitivity diseases in susceptible (atopic) subjects (Gravesen, 1979). There is also evidence suggesting that sensitization and exposure to fungi will increase the chances of asthma attack and some fungi are also clearly associated with symptoms of asthma (Salvaggio and Aukrust, 1981; Kauffman *et al.*, 1994). It is reasonable to hypothesize that the high fungal exposure in our region may play an important role in the development of asthma and contribute to its increasing prevalence.

In our investigation, the predominant genera in our region were *Cladosporium*, *Aspergillus*, *Penicillium*, *Alternaria*, and yeast. Total fungal concentrations with higher *Cladosporium* and *Aspergillus* occur in winter. *Cladosporium* often dominates the overall picture, inside as well as outside homes. *Cladosporium* is the most abundant genus identified from both indoor and outdoor samples (Solomon, 1975; Macher *et al.*, 1991; Ebner *et al.*, 1992; Shadzi *et al.*, 1993). In our region, the peak concentration occurred in winter and the lowest concentration in summer, and even the lowest concentration was still higher than observations from other countries. *Cladosporium* is also a well-known outdoor genus. According to the I/O ratio, and the strong correlation between indoor and outdoor concentrations throughout the year (Table 6), the indoor *Cladosporium* is expected to have come from outdoor penetration. Nevertheless the concentrations found were still too high to be neglected.

The genus *Aspergillus* also showed the highest concentration in winter and another peak concentration in summer. Although most houses had higher outdoor concentrations, the percentages of houses with a high I/O ratio of *Aspergillus* also increased from summer to winter. In addition, a significant indoor/outdoor correlation was found only in spring. Such a finding suggests that there are potential contaminated sources of *Aspergillus* in indoor environments in summer, fall, and winter. *Aspergillus* is a genus associated with mycotoxin-producing, allergic bronchopulmonary disease and other opportunistic infections (Hendry and Cole, 1993; Turner *et al.*, 1974; Gravesen, 1979). In addition, *Aspergillus*

versicolor, *A. flavus*, and *A. fumigatus* are so-called high-risk microorganisms and the confirmed presence of these species will require urgent risk management decisions to be made (AIHA, 1996). Therefore, the need for advanced and rapid analysis to identify the field samples at a species level is apparent.

Penicillium and *Alternaria* turn out to be present throughout the year, although in rather low concentrations compared to *Cladosporium*. Except for some dwellings, the indoor concentrations are also higher than those outdoors. *Alternaria* shows a peak concentration in spring and stays at generally low concentrations in other seasons. The I/O ratio and correlation between indoor and outdoor concentrations also indicate that most domestic *Alternaria* penetrate from outdoor environments. *Alternaria* is a group of common decay fungi and some investigations have documented that its peak concentration often occurs during crop harvesting and mowing (Moubasher *et al.*, 1990). The related activity disturbs the fungi in plants and incredible levels of spores will be dispersed into the atmosphere (Lacey, 1991). This may provide a reasonable explanation for the unusually high concentration of *Alternaria* in spring in our region because some sections of the city are covered with rice fields in which, for example, harvesting usually takes place at this time of the year. In addition, serial studies have reported that *Alternaria* is a fungus producing important allergens associated with asthma and other allergic diseases (Halonen, 1997). How the seasonal variations of *Alternaria* concentrations in homes affect the health of asthmatic and nonasthmatic children remains to be discovered in future studies.

In our investigation, overall fungal concentrations were generally higher indoors than outdoors, except for some houses contaminated with certain genera. Among them, *Aspergillus* and *Penicillium* are commonly genera with indoor sources. Being so-called soil fungi, they are also considered a group that is significantly associated with dirt-floor, crawl-space types of basements (Su *et al.*, 1992). In addition, since most fungal spores penetrated from outdoors in our region, the guideline to avoid the high concentration contributed from outdoors to indoors while still keeping the air exchange efficient is obviously an important subject.

We have tried to compare the fungal concentrations between homes of asthmatics and nonasthmatics in four different seasons. Our results do not find a consistent pattern between these two groups of homes for all fungi of interest. Only limited studies have supported the significant difference of

fungus exposure between asthmatic and nonasthmatic homes (Wickman *et al.*, 1992; Li and Hsu, 1995). A long-term, follow-up investigation to document how the changes of domestic airborne fungal exposure affect the severity of asthma in children and the development of respiratory illness or asthma by healthy children should be the focus of future endeavors.

CONCLUSIONS

The environmental microbial concentrations report in this study can be regarded as representative of the domestic exposure present in our region because study homes have been randomly selected, and the time period extends through an entire year, with the sampling frequency being once a month. In contrast with what has been observed in other studies, the *Der p 1* allergen concentration does not seem to vary with the fluctuation of relative humidity among the four seasons. However, our results show that about 65% of children's mattresses in southern Taiwanese homes have *Der p 1* allergen levels greater than 2 µg/g, the recommended level by WHO for inducing allergic reactions. Further studies are needed to verify the health effects on children with long-term, continuous exposure to such a high level of house dust mite allergens.

It is obvious that the domestic fungal concentrations in our region, in either asthmatic or nonasthmatic homes, are higher than many levels suggested to be health risks. In particular, potential contaminated sources of *Aspergillus* in indoor environments are suspected for every season except spring. The related mycotoxin effects, such as allergic bronchopulmonary diseases and other opportunistic infections, rightfully deserve much further concern. *Alternaria*, a famous allergen-producing fungus, shows a peak concentration in spring that may have been highly correlated with crop harvesting and mowing at that time in our region. Effective prevention through having children be away from excessive exposure to the contaminated environment should be among the top priorities.

We identify in this study that "season" seems to be a determinant factor for domestic endotoxin levels in these investigated houses. The concentrations reported are the first data of this kind for homes in a subtropical environment. We realize that much background data collection is still awaited before any threshold values can be proposed, while concurrent efforts should be made to appreciate the exact role of endotoxin in the etiology of allergic asthma.

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