The relationship between exposure to microbial volatile organic compound and allergy prevalence in single-family homes

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Abstract

Microbial volatile organic compounds (MVOCs) are a type of VOCs produced by microorganisms. Exposure to 1-octen-3-ol, one of the known MVOCs, has been reported to reduce nasal patency and increase nasal lavage myeloperoxidase, eosinophil cationic proteins, and lysozymes in both experimental and field studies. We reported in a previous paper that 1-octen-3-ol exposure at home is associated with mucosal symptoms. In this study, our aim was to investigate the relationship between asthma and allergies and MVOC exposure in single-family homes. The subjects were 624 inhabitants of 182 detached houses in six regions of Japan. Air samples were collected using diffusive samplers, and the concentrations of eight selected MVOCs were analyzed using gas chromatography/mass spectrometry in selected-ion-monitoring mode. Each inhabitant of each of the dwellings was given a self-administered questionnaire. Among the 609 subjects who answered all of the questions about allergies, history of the medical treatment for asthma, atopic dermatitis, allergic rhinitis, and allergic conjunctivitis within the past two years was 4.8%, 9.9%, 18.2%, and 7.1 %, respectively. A significant association between 1-octen-3-ol (per $\log_{10}$ unit) and allergic rhinitis odds ratio (OR): 4.10, 95% confidence interval (CI): 1.71 to 9.80 and conjunctivitis (OR: 3.54, CI: 1.17 to 10.7) was found after adjusting for age, sex, environmental-tobacco-smoke exposure, wall-to-wall carpeting in the home, signs of dampness, history of treatment for hay fever, and other potentially relevant environmental factors. No relationships were found between any MVOCs and asthma or atopic dermatitis after the adjustment. The levels of MVOCs and airborne fungi were only weakly correlated. These results are consistent with previous studies that have associated higher levels of 1-octen-3-ol exposure with increased irritation of nasal

and ocular mucosae. Although the indoor-air concentrations of 1-octen-3-ol found in this study were relatively low, we conclude that exposure to MVOC may be related to rhinitis and conjunctivitis.

Key words:

Microbial volatile organic compounds (MVOCs)

1-octen-3-ol

Home environment

Asthma

Allergies

1. Introduction

Asthma and allergies have been reported to be associated with dampness and/or the presence of fungi or moldy odors in indoor air (Bornehag et al., 2001; Bornehag et al., 2004; Walinder et al., 2001; Wieslander et al., 2007). Causative agents that have been suggested include mite-related allergens, microbiological exposures and chemicals emitted during the degradation of building materials, and the World Health Organization has published guidelines that include a comprehensive review of the scientific literature on health problems associated with moisture in buildings and with biological agents (WHO, 2009).

Microbial volatile organic compounds (MVOCs) include a range of chemicals produced by microorganisms as part of their metabolic processes; more than 200 compounds in this class have been discovered in chamber studies (Fiedler et al., 2001; Korpi et al., 1997; Pasanen et al., 1997; Scholler et al., 2002). MVOCs have been considered to be potential causative agents since Wessen and Schoeps (1996) reported having found 26 MVOCs whose concentrations were higher indoors than outdoors. MVOC concentrations are higher in buildings that have dampness problems caused by flooding than in control buildings (Wieslander and Norbäck, 2010). These results suggest that exposure to MVOCs is one of the possible effects of dampness.

Field studies measuring MVOC concentrations in relation to health outcomes are still scarce. When employees reoccupied buildings with high levels of MVOCs after flooding, increased signs of eosinophilic inflammation in the nasal mucosae were observed (Wieslander et al., 2007). Similar results were found among professional house painters, for whom exposed to higher levels of 1-octen-3-ol, a known MVOC, was related to reduced nasal patency and increases in nasal-inflammation biomarkers

(Wieslander and Norbäck, 2010). Two studies on MVOC exposure and asthma and allergies in schools found a relationship between MVOC exposure and an increased odds ratio (OR) for the occurrence of wheezing, nocturnal breathlessness, doctor-diagnosed asthma, and history of asthma among pupils and among employees (Kim et al., 2007; Smedje et al., 1996). There has been only one study of the home setting, which found that children living in dwellings with higher MVOC concentrations had a higher prevalence of asthma, hay fever, and wheezing, and the results were not statistically significant (Elke et al., 1999).

These results are supported by two experimental studies on humans that found significant objective and subjective signs of mild mucosal irritation in the eyes and airways during exposure to 3-methylfruran or 1-octen-3-ol over a period of 2 h in a closed chamber (Wålinder et al., 2005; Wålinder et al., 2008). Korpi et al. (1999) found that 1-octen-3-ol is more potent than other compounds and that its effect is stronger when combined with other compounds. According to a more recent review of the literature, the most obvious adverse health effects of MVOC exposure are eye and upper-airway irritation (Korpi et al., 2009). The exposure levels found in the chamber studies mentioned above were 1 mg/m^3 for 3-methylfuran and 10 mg/m^3 for 1-octen-3-ol (Wålinder et al., 2005; 2008). The recommended maximum level of 1-octen-3-ol for indoor air, based on data from animal studies, is estimated to be 100 $\mu g/m^3$ (Korpi et al., 1999). Actual average indoor MVOC concentrations in dwellings have been found to be up to 2 $\mu g/m^3$ (Araki et al., 2009; Elke et al., 1999; Matysik et al., 2009), which is far lower than those found in experimental studies and than the recommended maximum level. In response to these findings, one study suggested that the apparent relationship between health outcomes and MVOCs could be due to

exposure to the microorganisms themselves (Smedje et al., 1996). However, there are no studies that support this hypothesis.

There is only one study, at a school, that has reported both indoor MVOCs and measurable levels of viable mold measurements; a negative correlation was found (Kim et al., 2007). Although MVOCs have been related to odor perception (Keller et al., 2001; Műller et al., 2004a; Műller et al., 2004b), only a weak correlation has been found between MVOC concentrations and visible indoor mold (Schleibinger et al., 2008). In short, these studies failed to show that there is a relationship between MVOC levels and mold. However, compounds known to be MVOCs are also emitted by building materials themselves (Korpi et al., 1998; Pasanen et al., 1998). The levels of MVOCs were highest in the newest school studied (Kim et al., 2007), showing that other sources besides mold that emit MVOCs may be found indoors.

We have measured the levels of MVOCs in 182 detached houses(Araki et al., 2010). In our previous paper, we have reported that related concentrations of 1-octen-3-ol, in the home environment, to subjective reports of home-related mucous-production symptoms. Houses with condensation on both the walls and window panes had higher levels of 3-methyl-1-butanol than houses without such condensation. Levels of MVOCs were higher for wooden houses than for houses built from other materials, and there was no relationship between concentrations of MVOCs and signs of visible mold or moldy odors. The effects of MVOCs on inhabitants' asthma and allergies and correlations between MVOCs and concentrations of airborne fungi were not analyzed in that study. Therefore, the specific aim of the current study is to investigate the relationships between indoor MVOC levels in dwellings and recent prevalence of asthma and allergies. In addition, the relationships between MVOC

levels and viable airborne fungi were examined.

2. Methods

The details of the study design and methods of environmental measurements were described in our previous paper (Araki et al., 2010), so they are summarized only briefly here.

2.1 Study population

The subjects of the study were 624 inhabitants of 182 single-family homes in six regions of Japan, Sapporo, Fukushima, Nagoya, Osaka, Okayama and Fukuoka. This research is based on data collected in 2006. This was a partial second follow-up study, in which homes where MVOC measurements had been conducted in a first follow-up were revisited. In 2003, preliminary questionnaires on indoor-air quality had been sent to randomly selected single-family homes, which had all been constructed less than seven years ago; 2297 households responded (a response rate of 41.1%) (Kishi et al., 2009). Of the responding households, 425 agreed to home visits for environmental measurements in 2004 (Saijo et al., 2011; Takigawa et al., 2010). The first follow-up was conducted with 270 households in 2005 and the second follow-up was conducted with 182 households in 2006. The original study protocol was prospective; the subjects agreed to participate in the environmental measurements over three years. The resulting potential selection bias was analyzed by comparing the participants who continued with the study to those who did not, using the data from 2003 to 2004; there were no significant differences (Araki et al., 2010).

2.2 Questionnaire

Questionnaire survey and environmental measurements were conducted between September and December of 2006 (except for one set that was collected in January 2007). All of the inhabitants of each home were asked to fill out a self-administered questionnaire containing questions about age, gender, amount of time typically spent in the house, smoking status, and medical history. The medical outcomes studied were treatments for asthma and allergies within the preceding two years. Subjects who reported having received medical treatment for bronchial asthma, atopic dermatitis, allergic rhinitis, and/or allergic conjunctivitis at any time during the preceding two years were classified as positive. Medical treatment for hay fever and food allergies were also asked about; however, both of them were excluded from the outcomes studied because their specific causal agents are not among the indoor environmental variables measured in this study. However, treatment for hay fever was adjusted for in order to account for its effect on health outcomes. Parents filled in the questionnaires for inhabitants younger than six years old. Another questionnaire included questions about characteristics of the dwellings and living situations, such as the presence of environmental tobacco smoke (ETS), renovations within the preceding year, wall-to-wall carpeting, signs of dampness, pets in the home, duration of window opening each time (average of preceding one month), and frequency of mechanical-ventilation usage. Data from the preliminary questionnaire in 2003 were used for structural materials and the age of the building. Both the personal and the housing questionnaires were distributed and collected directly by the investigator who

visited each house to conduct environmental monitoring.

2.3 Environmental measurement of MVOCs and other factors

Air samples were collected in the room in each house where all of its inhabitants usually spent most of their time in order to determine participants' indoor exposure to chemicals, fungi, and dust-mite-related allergens. The methods we used to measure MVOC concentrations were described in detail by Araki et al. (2009, 2010). Briefly, to collect MVOCs, Supelco VOC-SD tube-type diffusive samplers containing carbon molecular sieves (Sigma-Aldrich, MO, USA) were set at a height of 100-150 cm from the floor and 100 cm from the wall for 48 h. Eight compounds were measured using gas chromatography/mass spectrometry in selected-ion-monitoring mode (GC/MS-SIM) (using a Hewlett-Packard 600N/MSD, Hewlett-Packard Co., CA, USA) at the Osaka Occupational Health Service Center, part of the Japan Industrial Safety and Health Association in Osaka, Japan. The limit of detection (LOD) was set at 0.25 $\mu g/m^3$; if concentrations were under the LOD, they were given a value of half the LOD.

Formaldehyde and VOC measurements were conducted using the method described by Takigawa et al. (Takigawa et al., 2004; 2010). Briefly, two types of tube-type diffusive samplers containing silica cartridges coated with 2,4-dinitrophenylhydrazine (DNPH), the Supelco DSD-DNPH (Sigma-Aldrich, MO, USA), and the Supelco VOC-SD (Sigma-Aldrich, USA), were used to collect formaldehyde and other VOCs, respectively. Each of the latter two types of samplers were placed in parallel with the MVOC samplers for 24 hours. The analysis of formaldehyde levels was conducted using a Hitachi D-7100 high-performance liquid

chromatograph equipped with a UV detector (Hitachi Ltd., Tokyo, Japan) at the Osaka Occupational Health Service Center in Osaka, Japan. The analysis of VOC levels was conducted via GC/MS (using a Hewlett-Packard 630N/MSD) at the Kanto Occupational Health Service Center, part of the Japan Industrial Safety and Health Association in Tokyo, Japan. When formaldehyde and VOC concentrations were lower than their respective LODs of 5 μ/m^3 and 10$\mu g/m^3$, they were given a value of half the LOD. The sum of the results for the 29 VOCs tested for were treated as a single factor (Araki et al., 2010).

To determine what species of airborne fungi were present, air was collected using an AINEX BIO-SAS sampler (International PBI S.p.A., Milano, Italy) with a nine-centimeter petri dish containing dichloran-18% agar (DG-18) with 100 μg/l chloramphenicol with an airflow rate of 100 l/min for 1 min. The identification and counting of fungal species were conducted at the Mitsubishi Chemical Medience Corporation in Tokyo, Japan. In addition to individual genera of fungi, the total number of identified colonies was considered as a single factor, measured in colony-forming units, or CFU/m^3. In transforming data to logarithmic values, we gave samples with no colonies a value of 0.5 CFU/m^3. This method for measuring fungal concentrations has been described by Takahashi (1997) and Saijo et al. (2001).

To determine dust-mite-related allergen levels, the method described by Ogino et al. (2000) and Saijo et al. (2011) was used. Floor dust samples were collected with an HC-V15 hand vacuum cleaner (National, Osaka, Japan). Levels of *Dermatophagoides pteronyssinus* allergens (Der p1) and *Dermatophagoides farinae* allergens (Der f1) were determined by enzyme-linked immunosorbent assays (ELISAs) (using Der p1 and Der f1 ELISA kits, Nichinichi Pharmaceutical Co., Ltd., Mie, Japan).

When allergen levels were lower than the LOD of 0.1 μg/g of fine dust, they were given a value of 0.05 μg/g. The sum of the values for Der p1 and Der f1 were combined into a single factor, called Der1.

2.4 Data analysis

To determine the relationships between the prevalence of asthma and allergies and the concentrations of MVOCs, formaldehyde, VOCs, fungi, and mite-related allergens, a statistical analysis was conducted by logistic regression and the ORs with 95% confidence intervals (95%CIs) were calculated. Signs of dampness were used to construct an index. When the subjects reported visible mold growth, condensation, moldy odor, high humidity in bathrooms, or water leakage within the preceding five years, these were each defined as positive signs of dampness. The dampness index (0-5) was calculated by summing the number of signs of dampness out of those five (Kishi et al., 2009; Saijo et al., 2011).

Because their distributions were right-skewed, each of the environmental variables was $\log_{10}$-transformed before analysis. First, crude ORs and 95%CIs were calculated, and then they were adjusted for gender, age group (divided into 15-year blocks), tobacco smoking or ETS exposure (categorical variable as smoker, nonsmoker with ETS, nonsmoker without ETS), wall-to-wall carpeting in the home, renovation within the preceding year, dampness index, and history of medical treatment for hay fever. The effects of each environmental variable were modeled separately. Finally, all of the environmental variables whose effects were significant at $p<0.1$ in the initial models were modeled together, and the model was then adjusted as mentioned SPSS for

Windows version 14.0J (SPSS Inc., IL, USA) was used for the analysis; a 5% significance level was applied.

2.5 Ethical considerations

The study protocol was approved by the ethical board for epidemiological studies at the Hokkaido University Graduate School of Medicine and by the ethical boards at all of the regional universities involved in the study. All of the subjects and, when relevant, their parents gave written informed consent to participate in the study.

3. Results

Among the 624 subjects, 609 answered all of the questions about allergies completely and were therefore included in the analysis. Table 1 summarizes the demographic data, smoking status and history of asthma and allergy treatment of the study subjects. The prevalence of asthma, atopic dermatitis, allergic rhinitis, and allergic conjunctivitis within the preceding two years was 4.8%, 9.9%, 18.2%, and 7.1%, respectively. The prevalence of hay-fever treatment within the preceding two years was 16.3%.

Table 2 shows the relationships between asthma and allergies and the subjects' personal and living-space characteristics. All of the symptoms were more prevalent in the younger age groups, but there were no differences between males and females. The frequency of alcohol consumption was lower among allergic subjects. The prevalence of medical treatment for allergic rhinitis and conjunctivitis was significantly

higher among subjects who had hay fever, and those who reported hay fever among subjects with rhinitis and conjunctivitis were 57% and 21%, respectively. The housing characteristics that were significantly or marginally related to medical outcomes were having undergone renovation within the preceding year, having wall-to-wall carpeting and showing signs of dampness such as visible mold growth, condensation or high humidity in the bathroom.

The levels of chemical and biological contaminants found are given in Table 3. The most-concentrated and most-frequently-detected MVOC was 1-pentanol, with a median concentration of 0.60 $\mu g/m^3$ and a detection rate above the LOD of 78.6%. There were no dwellings in which a 3-octanol concentration above the LOD was detected. The most prevalent fungal genus detected was *Cladosporium*; its mean percentage of the total fungal culture was 52.6%. The five most-prevalent fungal genera, *Aspergillus*, *Cladosporium*, *Cryptococcus*, *Rhodotorula* and *Penicillium* together made up an average of 81.5% of the total fungi.

Table 4 shows the relation between the levels of MVOC and other environmental variables, and duration of window opening each time. Homes where windows were open for longer periods each time (>1 hour) had lower levels of MVOCs, formaldehyde, and VOCs, whereas higher levels of *Cladosporium*. Smaller ratios of sum of fungi except *Cladosporium* to *Cladosporium* were seen in homes where windows were open for longer periods. The levels of other fungal genera besides *Cladosporium,* and mite-related allergens were not significantly correlated with the duration of window opening.

Table 5 shows the correlations between the MVOCs found and the airborne fungal genera. It also shows that there were positive correlations among the MVOCs.

There were weak positive correlations between the levels of 1-pentanol and the prevalence of *Aspergillus* and between the levels of 2-hexanol and the prevalence of *Cryptococcus* and *Rhodotorula*. In contrast, there were weak negative correlations between the levels of MVOCs and the prevalence of *Cladosporium*. The ratio of the prevalence of sum of other fungal genera *except Cladosporium* to the prevalence of *Cladosporium* was calculated, and it was found to be weakly but positively correlated with MVOC concentrations.

Table 6 shows the effects of environmental factors on allergy prevalence, giving both the unadjusted and the adjusted statistics. After adjustment, positive relationships were found between the prevalence of atopic dermatitis and 2-hexanone and 1-octen-3-ol, between allergic rhinitis and 1-pentanol, 2-hexanone, and 1-octene-3-ol, and between allergic conjunctivitis and 1-octen-3-ol. The total concentration of fungi was negatively associated with atopic dermatitis, allergic rhinitis, and allergic conjunctivitis. Asthma was not found to be related either MVOC concentrations nor the other environmental factors studied. The ratio of sum of fungi except *Cladosporim* to *Cladosporium* was positively correlated with atopic dermatitis (OR: 1.06, 95%CI: 1.02-1.11, p=0.007), allergic rhinitis (OR: 1.05, 95%CI: 1.01-1.09, p=0.019), and allergic conjunctivitis (OR: 1.07, 95%CI: 1.01-1.13, p=0.016) after adjustment (ORs were expressed as every steps of 10). (This data is not given in the tables.)

After mutual adjustment among all of the environmental variables that were significantly or marginally related to any of the symptoms studied ($p<0.1$), 1-octen-3-ol was found to be positively correlated with allergic rhinitis and conjunctivitis, as shown in table 6. The total concentration of airborne fungi was negatively correlated with

atopic dermatitis and allergic conjunctivitis.

4. Discussion

This study found that the level of 1-octen-3-ol in a dwelling was related to inhabitants' history of medical treatment for allergic rhinitis and conjunctivitis after mutual adjustment among multiple environmental variables. The definition of allergic rhinitis in this study covers both seasonal and perennial allergic rhinitis. Therefore, to analyze the relationship between MVOCs and asthma and allergies, the models were adjusted to account for hay fever. The prevalence of rhinitis, conjunctivitis and hay fever were 18.2%, 7.1%, and 16.3%, respectively. Skoner (2001) has reported that the prevalence of rhinitis in the general population ranges from 3% to 19% and that that prevalence of seasonal allergic rhinitis (hay fever) is around 10%. According to the nationwide survey conducted in Japan, estimated prevalence of Japanese cedar pollinosis was 13.1% (Okada 2003). The prevalence of rhinitis and hay fever found in the present study were slightly higher than those described by Skoner and Okada. This may be due to the study protocol; the subjects in this study agreed to participate for three years, and it may be that individuals with more severe symptoms may be more willing to be involved in a health study (Bornehag et al., 2006). According to Sakashita et al., (2008), the most common allergen in allergic rhinitis among Japanese subjects between 20-49 years of age was Japanese cedar pollen (86% with allergic rhinitis) followed by mites. Fifty seven percent of those who reported rhinitis, and 21% of those who reported conjunctivitis also reported hay fever in this study (Table 2). In general, adjusting for dependent variables too closely related would weaken

associations. However, interestingly, the relationship between MVOCs and rhinitis and conjunctivitis were significant with bigger ORs when hay-fever was included in dependent variables than without.

In experimental studies with human participants, higher levels of the nasal-lavage biomarkers eosinophil cationic protein, myeloperoxidase and lysozyme were found after participants were exposed to 1-octen-3-ol (Walinder et al., 2008). In addition, Wieslander and Norback (2010) found in a field study that higher levels of 1-octen-3-ol exposure were associated with higher levels of nasal lavage myeloperoxidase and lower nasal patency among painters. In a previous paper, we reported that 1-octen-3-ol was related to study participants' subjective reports of home-related mucous symptoms, ocular problems, nasal problems, dry throats and coughing (Araki et al., 2010). In an animal study, Korpi et al. (1999) found that, of the 11 MVOCs they investigated, 1-octen-3-ol required the lowest concentration to cause a 50% decrease in respiratory rate (RD50). In addition, the molecular structure of 1-octen-3-ol has a hydroxyl close to unsaturated hydrocarbons; double-bonded π electrons easily conjugates with alcohol, and may form more-irritating end products (Carslaw, 2003; Wolkoff et al., 1999; 2006). Therefore, 1-octen-3-ol may be a more potent compound in terms of causing adverse health effects. The results of this study are consistent with previous studies that have found that higher levels of 1-octen-3-ol exposure are related to irritation of the nasal and ocular mucosae and thus to the need for the medical treatment for allergic rhinitis and conjunctivitis. However, the concentrations tested in experimental exposure studies were much higher than those we found in the single-family homes in our study.

When the effects of 1-pentanol, 2-hexanone, 2-heptanone, and 1-octen-3-ol

were modeled separately, they were found to be correlated with the prevalence of atopic dermatitis. However, none of them were found to be correlated with atopic dermatitis after mutual adjustment among the variables. In Elke et al study (1999), higher ORs were observed for the relationship between the prevalence of asthma, hay fever, and irritation of the eyes and higher levels of 3-methyl-1-butanol and 1-octen-3-ol, although they were not statistically significant; in contrast, none of the MVOCs Elke studied were found to be related to eczema nor itchy skin rashes. There have been no other studies that examine the relationship between MVOC exposure and dermal symptoms.

Although allergic rhinitis is a risk factor for asthma (Leynaert et al., 2000), levels of MVOCs were not correlated with asthma in this study. In this study, having medical treatments for asthma at anytime during the preceding two years was treated as positive, but not current asthma symptoms such as asthma attacks, wheeze, and/or attacks of breathlessness as previous paper. Kim et al. (2007) reported that several MVOCs are related to nocturnal breathlessness, wheezing and doctor-diagnosed asthma but that the relationships between MVOCs and wheezing and doctor-diagnosed asthma seem weaker than the relationship between MVOCs and nocturnal breathlessness. Korpi et al. (2009) suggested that the most obvious health effects of MVOC exposure are eye and upper-airway irritation.

In this study, total concentrations of fungi were negatively correlated with allergy-symptom history. A similar result was found in Kim et al (2007) study; the author suggested that the findings may have been the result of differences in window- or door-opening habits. We asked participants about their daily window-opening habits by questionnaire. To find the relationship between window-opening habits and levels of MVOC and fungi, we have calculated the ratio of sum of fungi except *Cladosporium*

to *Cladosporium.* We found that homes where windows were open for longer periods each time had higher levels of *Cladosporium* and smaller ratio of sum of fungi except *Cladosporium* to *Cladosporium* than homes where windows were open for shorter periods. The levels of other fungal genera besides *Cladosporium* were not significantly correlated with the duration of window opening. Kim et al. (2007) showed that the levels of viable *Cladosporium* were higher outdoors than indoors, whereas the levels of *Penicillium* were higher indoors than outdoors. According to Sautour et al. (2009), *Cladosporium* was a dominant genus outdoors from spring to autumn. Majority of the environmental examination in this study was conducted in autumn season. As a result, the airborne *Cladosporium* levels were significantly affected by windows being open, but levels of fungi beside *Cladosporium* were not.

The fact that the concentrations of MVOCs were correlated with each other suggests that they are emitted from the same source. The finding that levels of MVOCs showed a weak negative correlation with *Cladosporium* concentrations may be confounded with the length of time windows were left open in the households studied. Meanwhile, the levels of 2-pentanol and of *Aspergillus* and the levels of 2-hexanone and of *Cryptococcus* and *Rhodotorula* were only weakly positively correlated. Five compounds of MVOCs and sum of fungi except *Cladosporium* to *Cladosporium* were also positively correlated, but correlation coefficients were small. Therefore, contribution of airborne fungi as an emission source for MVOCs may therefore be very small. Other reason is that some part of MVOC may be from hidden microbial growth inside the construction. There have so far been no other studies that examine the levels of MVOCs and airborne fungal genera in indoor air. In our previous paper on this research, we reported that levels of 2-hexanone and 2-heptanone were not correlate

with the presence of visible mold nor moldy odors (Araki et al., 2010). Other researchers have reported similar results and have found that MVOCs were being emitted from sterilized building materials (Korpi et al., 1998; Schleibinger et al., 2008). It is therefore possible that the building materials and other such sources could be the source of the compounds. For example, linoleum flooring, which contains linoleic acid, has been shown to be a source of 1-octen-3-ol (Husson et al., 2002; Wurzenberger and Grosch, 1984). However, 79% of the dwellings in our study had wood floors, and none of them had linoleum floors.

The question remains regarding whether or not 1-octen-3-ol itself increases the risk of allergic reaction. The level of 1-octen-3-ol found in our field study was far lower than that used in earlier experimental studies with humans and animals (Korpi et al., 1999; Wålinder et al., 2008). It may be that 1-octen-3-ol is an indicator of indoor mold growth, which causes allergic symptoms (Smedje et al., 1996). We found that the ratio of sum of fungi except *Cladosporim* to *Cladosporium* was positively correlated with atopic dermatitis, allergic rhinitis, and allergic conjunctivitis. That is to say, when there was a higher prevalence of fungi beside *Cladosporium* in indoor air, the prevalence of atopic dermatitis, allergic rhinitis and conjunctivitis was higher than when there was a higher prevalence of *Cladosporium*. The relationships between 1-octen-3-ol and allergic rhinitis and conjunctivitis did not differ from the results given in table 7 when the model was adjusted to include the ratio of sum of fungi except *Cladosporium* to *Cladosporium.* Therefore, we can conclude that the correlations between 1-octen-3-ol and allergic rhinitis and conjunctivitis are independent of both the overall prevalence of viable airborne fungi and the ratio of sum of fungi except *Cladosporium* to *Cladosporium*.

This study has several limitations. First, the subjects may have confused allergic and nonallergic rhinitis in their answers, despite the fact that the questionnaire asked about diagnoses of allergic rhinitis and conjunctivitis. It is possible that misclassification of the rhinitis, conjunctivitis and pollen allergy could also have occurred due to an overestimation of the actual frequency of rhinitis and conjunctivitis because of the confounding effects of pollen. In addition, we did not gather information about the severity of the conditions or on the frequencies of medical treatment. We did not use laboratory tests, such as detecting allergen-specific immunoglobulin E (IgE) antibodies, to determine participants' allergies. However, an IgE antibody test is not by itself sufficient for a diagnosis of allergy (Skoner, 2001). Misclassification of the medical outcomes under investigation could have occurred; possibly due to an underestimation of the actual frequency with which rhinitis occurs (Skoner, 2001).

Secondly, it is difficult to clinically diagnose asthma and allergies in younger children. However, we conducted an analysis that only included the subjects above the age of six, which is the youngest age specified by the International Study of Asthma and Allergies in Childhood (ISAAC) (Strachan et al., 1997), and the results followed the same trend as that shown in Table 5. We can therefore discount the possible effects of misdiagnosis of toddlers, and the findings can include all of the subjects.

Thirdly, there are many factors that cause rhinitis and conjunctivitis, and it is not possible to adjust for all of the potential confound. Tests for environmental exposures were only performed in participants' homes; potential exposure levels at their offices or schools were not considered. However, some known environmental factors that affect allergies, such as wall-to-wall carpeting, recent renovations, and dampness in

the home were adjusted for, and the levels of dust-mite allergens were not found to be related to the symptoms studied. Therefore, the associations we found between 1-octen-3-ol and allergies can be assumed to be independent of other home-related environmental factors.

Fourthly, the study was cross-sectional in design, so causal relationships cannot be determined. However, the medical outcomes we studied were defined by subjects' medical treatment within the preceding two years, and each subject had been living in the same house for more than two years. The chance of significant changes in subjects' home environments can therefore be discounted.

Finally, the environmental variables were measured only once in this phase of the study, and sampling period is very short. Therefore, levels of airborne fungi do not represent overall levels of fungi growing indoors. Other methods should be used in future studies to measure levels of fungi indoors, such as dust sampling or wall swabs.

5. Conclusion

The research presented in this paper is based on a field study that measured MVOC concentrations and levels of airborne indoor fungi. The results show positive correlations between levels of 1-octen-3-ol and prevalence of allergic rhinitis and conjunctivitis as measured by a history of medical treatments within the two years preceding the study. These correlations were found to hold independent of other home environmental factors that may cause allergy symptoms, of the levels of fungi in the homes studied, and of subjects' prevalence of hay fever. The levels of MVOCs and of airborne fungi were only weakly correlated, leading the researchers to suspect that airborne fungi are not a significant source of airborne MVOCs. The levels of MVOCs

we found indoors in this field study were far lower than those tested in experimental studies, suggesting that the direct adverse allergic effects found from those compounds in those previous studies should be further examined. Additional studies are needed to clarify the actual effects of low concentrations of MVOCs.

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Table 1 Personal characteristics and prevalence of allergy-treatment history (N=609)

	Total		Males		Females	
	N	(%)	N	(%)	N	(%)
Gender						
Male	296	48.6				
Female	313	51.4				
Age group						
0-14	161	26.4	81	27.4	80	25.6
15-29	31	11.2	37	12.5	31	9.9
30-44	96	26.8	67	22.6	96	30.7
45-59	60	20.4	64	21.6	60	19.2
60+	46	15.3	47	15.9	46	14.7
Tobacco-smoking status						
Current smoker	54	8.9	43	14.5	11	3.5
Nonsmoker, ETS exposure at home	82	13.5	26	8.8	56	17.9
Nonsmoker, no-ETS exposure at home	473	77.7	227	76.7	246	78.6
Allergy history						
Asthma	29	4.8	18	6.1	11	3.5
Atopic dermatitis	60	9.9	34	11.5	26	8.3
Allergic rhinitis	111	18.2	50	16.9	61	19.5
Allergic conjunctivitis	43	7.1	19	6.4	24	7.7
Hay fever	102	16.7	49	16.6	53	16.9

Table 2 Relationships between asthma and allergy-treatment history and personal and living-space characteristics (N=609)

Factors	Values	n	Asthma		Atopic dermatitis		Allergic rhinitis		Allergic conjunctivitis	
			(%)	p	(%)	p	(%)	p	(%)	p
Personal characteristics										
Gender	Male	296	6.1	0.182	11.5	0.221	16.9	0.462	6.4	0.636
	Female	313	3.5		8.3		19.5		7.7	
Age group	**0-14**	**161**	**11.2**	**<0.001**	**19.9**	**<0.001**	**26.7**	**0.001**	**11.8**	**0.017**
	15-29	**68**	**4.4**		**20.6**		**22.1**		**8.8**	
	30-44	**163**	**1.8**		**4.3**		**17.2**		**7.4**	
	45-59	**124**	**3.2**		**4.0**		**15.3**		**3.2**	
	60+	**93**	**1.1**		**2.2**		**6.5**		**2.2**	
Smoking status	Smoker	54	3.7	0.797	**0.0**	**0.030**	14.8	0.087	1.9	0.251
	Nonsmoker, ETS	82	3.7		**8.5**		26.8		7.1	
	Nonsmoker, no-ETS	473	5.1		**11.2**		17.1		7.8	
Frequency of alcohol consumption	≧Once/week	220	1.9	0.059	6.6	0.202	16.6	0.128	**3.3**	**0.002**
	< Once/week	337	4.8		9.0		19.9		**9.9**	
Hay fever	Yes	102	5.9	0.609	13.7	0.149	**56.9**	**<0.001**	**20.6**	**<0.001**
	No	507	4.5		9.1		**10.5**		**4.3**	
Time spent in the house	17h +	214	5.1	0.652	8.4	0.416	18.7	0.860	5.1	0.188
	< 17h	394	4.3		10.5		18.1		8.2	
Housing characteristics										
Structural material	Wood	473	4.2	0.211	9.5	0.512	19.5	0.134	7.2	0.992
	Others	131	6.9		11.5		13.7		6.9	
Age of house	3-5 years	526	4.6	0.510	9.5	0.403	18.6	0.432	7.6	0.221
	6-8 years	80	6.3		12.5		15.0		3.8	
Renovation within preceding year	Yes	23	**21.7**	**<0.001**	21.7	0.051	30.4	0.122	**21.7**	**0.005**
	No	586	**4.1**		9.4		17.7		**6.5**	
Wall-to-wall carpeting	Yes	22	4.5	0.961	18.2	0.182	22.7	0.578	**18.2**	**0.038**
	No	587	4.8		9.5		18.1		**6.6**	
Visible mold growth	Yes	492	4.9	0.783	9.6	0.611	19.5	0.092	**8.3**	**0.012**
	No	117	4.3		11.1		12.8		**1.7**	
Condensation	Yes	419	**6.0**	**0.040**	10.3	0.641	20.3	0.057	8.1	0.140
	No	188	**2.1**		9.0		13.8		4.8	
Moldy odor	Yes	118	3.9	0.519	10.2	0.919	19.5	0.720	10.2	0.124
	No	487	4.5		9.9		18.1		6.2	
High humidity in the bathroom	Yes	122	6.6	0.305	9.0	0.714	23.8	0.081	8.2	0.596
	No	484	4.3		10.1		16.9		6.8	
Water leakage within preceding 5 years	Yes	68	7.4	0.291	11.8	0.581	11.8	0.149	4.4	0.362
	No	539	4.5		9.6		18.9		7.4	
Pets in the dwelling	Yes	206	2.9	0.118	7.3	0.117	20.9	0.254	7.8	0.656
	No	398	5.8		11.3		17.1		6.8	
Duration of window opening	<30 minutes	255	5.1	0.642	5.1	0.642	23.5	0.005	7.8	0.518
	> 1 hour	327	4.3		4.3		14.4		6.4	
Mechanical-ventilation usage	Always/often occasionally/	236	5.1	0.841	12.3	0.114	19.5	0.598	7.6	0.753
	Never/no ventilation	360	4.7		8.3		17.8		6.9	

The p-values were calculated using chi-square tests.

Table 3 Distribution of MVOC concentrations and levels of other environmental variables (N=182)

	Min	25%	50%	75%	Max	>LOD (%)
MVOCs (μg/m^3)						
3-Methyl-1-butanol	<LOD	<LOD	0.49	1.12	10.64	68.7
1-Pentanol	<LOD	0.28	0.60	1.47	12.15	78.6
2-Pentanol	<LOD	<LOD	<LOD	0.63	4.17	48.4
2-Hexanone	<LOD	<LOD	0.33	0.53	2.56	70.9
2-Heptanone	<LOD	<LOD	<LOD	0.29	1.52	35.2
3-Octanone	<LOD	<LOD	<LOD	<LOD	1.88	7.7
3-Octanol	<LOD	<LOD	<LOD	<LOD	<LOD	0.0
1-Octene-3-ol	<LOD	<LOD	<LOD	0.28	8.58	29.1
Formaldehyde (μg/m^3)	<LOD	21.3	32.6	46.9	120.1	99.5
Total for 29 VOCs (μg/m^3)	<LOD	35.1	76.1	148.1	2,798.9	85.2
Total fungi (CFU/m^3)	0	150	330	550	3,490	98.9
Cladosporium	*0*	*40*	*190*	*382*	*3340*	*88.5*
Aspergillus	*0*	*0*	*10*	*20*	*950*	*51.6*
Cryptococcus	*0*	*0*	*0*	*0*	*80*	*9.9*
Rhodotorula	*0*	*0*	*0*	*10*	*150*	*29.1*
Penicillium sp.	*0*	*10*	*20*	*50*	*2210*	*78.0*
All the rest	*0*	*20*	*40*	*80*	*1360*	*86.8*
Der1 mite-related allergens (μg/g of fine dust)	<LOD	0.53	2.47	8.83	502.27	91.2

Table 4 MVOC concentrations and levels of other environmental variables and duration of window opening

	<30 min			> 1hour			p
	25%	50%	75%	25%	50%	75%	
MVOCs (µg/m^3)							
3-Methyl-1-butanol	0.36	0.84	1.62	<LOD	0.30	0.69	<0.001
1-Pentanol	0.37	0.91	2.01	<LOD	0.48	1.00	0.001
2-Pentanol	<LOD	0.35	0.72	<LOD	<LOD	0.44	0.005
2-Hexanone	0.28	0.41	0.63	<LOD	0.28	0.45	<0.001
2-Heptanone	<LOD	<LOD	0.35	<LOD	<LOD	0.26	0.004
3-Octanone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.706
1-Octene-3-ol	<LOD	<LOD	0.43	<LOD	<LOD	<LOD	<0.001
Formaldehyde (µg/m^3)	25.5	36.45	52.8	18.4	28.8	44.9	0.032
Total for 29 VOCs (µg/m^3)	63.7	123.2	326.0	25.9	54.2	103.1	<0.001
Total fungi (CFU/m^3)	90.0	180.0	487.5	290.0	430.0	600.0	<0.001
Cladosporium	2.5	50	197.5	160	270	430	<0.001
Aspergillus	0	10	17.5	0	10	20	0.847
Cryptococcus	0	0	0	0	0	0	0.253
Rhodotorula	0	0	10	0	0	10	0.502
Penicillium sp.	10	20	50	10	20	40	0.697
All the rest	10	35	87.5	20	50	80	0.181
Sum of fungi except *Cladosporium* /*Cladosporium*[a]	0.6	2.0	18.5	0.16	0.35	0.87	<0.001
Der1 mite-related allergens (µg/g of fine dust)	0.6	1.95	5.42	0.435	2.59	10.105	0.631

The p-values were calculated using Mann-Whitney U tests.

[a], Sum of fungi except Cladosporium/Cladosporium is a ratio of "sum of fungal genera colony numbers except Cladosporium" to Cladosporium.

Table 5 Correlations between MVOC concentrations and concentrations of airborne fungi (N=182)

	3-Methyl-1-butanol	1-Pentanol	2-Pentanol	2-Hexanone	2-Heptanone	3-Octanone	*Cladosporium*	*Aspergillus*	*Cryptococcus*	*Rhodotorula*	*Penicillium sp.*	Sum of fungi except *Cladosporium* /*Cladosporium*[a]
3-Methyl-1-butanol							-0.220**	0.127	0.101	0.102	0.004	0.258**
1-Pentanol	0.280**						-0.235**	0.087	0.099	0.115	0.038	0.224**
2-Pentanol	0.390**	0.163*					-0.239**	0.191**	0.111	0.062	0.002	0.269**
2-Hexanone	0.403**	0.759**	0.214**				-0.279**	0.104	0.174*	0.186*	-0.038	0.269**
2-Heptanone	0.280**	0.676**	0.169*	0.719**			-0.269**	0.084	0.129	0.112	0.017	0.277**
3-Octanone	0.099	0.111	0.201**	0.066	0.206**		-0.090	0.062	0.035	0.001	0.079	0.064
1-Octene-3-ol	0.261**	0.421**	0.271**	0.427**	0.420**	0.133	-0.269**	0.012	0.129	-0.003	0.027	0.229**

The correlations were measured in terms of Spearman's rank correlation coefficients.

[a], Sum of fungi except *Cladosporium*/*Cladosporium* is a ratio of "sum of fungal genera colony numbers except *Cladosporium*" to *Cladosporium.*

* $p<0.05$

** $p<0.01$

Table 6 Relationships between asthma and allergy-treatment history and environmental variables

	Asthma			Atopic dermatitis			Allergic rhinitis			Allergic conjunctivitis		
Unadjusted	OR	95%CI	p	OR	95%CI	p	OR	95%CI	p	OR	95%CI	p
MVOCs												
3-Methyl-1-butanol	1.10	0.49-2.45	0.819	0.70	0.39-1.26	0.239	1.22	0.78-1.89	0.387	0.81	0.41-1.60	0.546
1-Pentanol	1.80	0.82-3.95	0.145	**1.83**	**1.04-3.22**	**0.036**	1.47	0.96-2.27	0.079	1.64	0.85-3.15	0.139
2-Pentanol	0.90	0.39-2.11	0.816	1.00	0.55-1.81	0.991	1.14	0.73-1.80	0.558	1.07	0.54-2.13	0.842
2-Hexanone	2.53	0.78-8.27	0.123	**2.41**	**1.03-5.63**	**0.042**	1.80	0.94-3.46	0.076	1.83	0.69-4.86	0.228
2-Heptanone	1.16	0.30-4.49	0.832	2.31	0.94-5.69	0.069	1.56	0.75-3.25	0.233	0.99	0.31-3.13	0.982
3-Octanone	1.37	0.25-7.65	0.717	1.91	0.61-5.93	0.265	0.57	0.16-1.98	0.374	0.08	0.00-4.44	0.215
1-Octene-3-ol	1.71	0.58-5.09	0.332	**2.13**	**0.99-4.59**	**0.053**	**1.98**	**1.06-3.70**	**0.032**	2.29	0.96-5.44	0.061
Formaldehyde	1.81	0.44-7.36	0.408	1.08	0.40-2.89	0.885	**2.18**	**1.00-4.76**	**0.049**	2.78	0.85-9.06	0.090
Total for 29 VOCs	0.86	0.16-4.64	0.858	0.90	0.27-2.94	0.855	0.76	0.29-1.94	0.562	0.24	0.04-1.55	0.135
Total fungi	0.62	0.29-1.29	0.199	**0.40**	**0.24-0.67**	**0.001**	0.69	0.45-1.05	0.083	**0.39**	**0.22-0.70**	**0.002**
Der1 mite-related allergens	0.95	0.60-1.49	0.820	0.96	0.69-1.32	0.789	0.98	0.76-1.26	0.878	1.05	0.72-1.52	0.798
Adjusted[a]	OR	95%CI	p	OR	95%CI	p	OR	95%CI	p	OR	95%CI	p
MVOCs												
3-Methyl-1-butanol	1.04	0.44-2.49	0.925	0.74	0.39-1.38	0.340	1.30	0.77-2.20	0.317	0.74	0.35-1.53	0.410
1-Pentanol	1.44	0.62-3.35	0.398	1.81	0.98-3.33	0.057	**1.81**	**1.08-3.05**	**0.025**	1.33	0.65-2.71	0.437
2-Pentanol	0.98	0.38-2.50	0.966	1.13	0.59-2.15	0.714	1.41	0.82-2.42	0.209	1.24	0.59-2.61	0.571
2-Hexanone	2.11	0.57-7.84	0.263	**2.71**	**1.07-6.84**	**0.035**	**2.38**	**1.07-5.27**	**0.032**	1.48	0.47-4.63	0.499
2-Heptanone	0.85	0.20-3.52	0.820	2.41	0.89-6.51	0.084	1.83	0.77-4.33	0.170	0.67	0.18-2.44	0.543
3-Octanone	1.56	0.23-10.5	0.648	2.79	0.81-9.61	0.104	0.62	0.16-2.39	0.490	0.08	0.01-7.93	0.285
1-Octene-3-ol	1.94	0.55-6.83	0.303	**2.64**	**1.12-6.20**	**0.026**	**5.00**	**2.36-10.6**	**<0.001**	**4.80**	**1.72-13.4**	**0.003**
Formaldehyde	1.15	0.26-508	0.606	1.08	0.37-3.10	0.738	2.14	0.85-5.40	0.107	2.21	0.60-8.24	0.236
Total for 29 VOCs	1.19	0.19-7.36	0.830	1.37	0.38-4.91	0.627	2.09	0.73-5.95	0.167	0.38	0.05-2.72	0.337
Total fungi	0.59	0.26-1.35	0.231	**0.33**	**0.18-0.61**	**<0.001**	**0.51**	**0.31-0.84**	**0.009**	**0.30**	**0.15-0.60**	**0.001**
Der1 mite-related allergens	1.07	0.64-1.81	0.867	1.07	0.73-1.57	0.595	1.24	0.91-1.69	0.178	1.20	0.77-1.85	0.422

Each environmental variable was modeled separately using a logistic-regression model.
The odds ratios were calculated using $\log_{10}$-transofrmed variables.
[a] The model was adjusted for gender, age, tobacco smoke exposure, renovation history, wall-to-wall carpeting, dampness index, and hay-fever.

Table 7 Relationships between allergy-treatment history and selected environmental variables

	Atopic dermatitis			Allergic rhinitis			Allergic conjunctivitis		
	OR	95%CI	p	OR	95%CI	p	OR	95%CI	p
1-Pentanol	1.08	0.42-2.79	0.878	1.02	0.46-2.28	0.964	-		
2-Hexanone	1.72	0.36-8.27	0.499	1.12	0.33-3.75	0.856	-		
2-Heptanone	0.83	0.19-3.73	0.811	-			-		
1-Octene-3-ol	1.63	0.55-4.85	0.379	**4.10**	**1.71-9.80**	**0.002**	**3.54**	**1.17-10.7**	**0.026**
Total fungi	**0.07**	**0.02-0.70**	**0.002**	0.64	0.38-1.08	0.097	**0.36**	**0.18-0.74**	**0.005**

All of the variables were modeled together in a logistic-regression model.
The odds ratios were calculated using $\log_{10}$-transformed variables.
The model was adjusted for gender, age, tobacco smoke exposure, renovation history, wall-to-wall carpeting, dampness index, and hay fever.