

Indoor molds, bacteria, microbial volatile organic compounds and plasticizers in schools – associations with asthma and respiratory symptoms in pupils

Abstract We investigated asthma and atopy in relation to microbial and plasticizer exposure. Pupils in eight primary schools in Uppsala (Sweden) answered a questionnaire, 1014 (68%) participated. Totally, 7.7% reported doctor-diagnosed asthma, 5.9% current asthma, and 12.2% allergy to pollen/pets. Wheeze was reported by 7.8%, 4.5% reported daytime breathlessness, and 2.0% nocturnal breathlessness. Measurements were performed in 23 classrooms (May–June), 74% had <1000 ppm CO₂ indoors. None had visible mold growth or dampness. Mean total microbial volatile organic compound (MVOC) concentration was 423 ng/m³ indoors and 123 ng/m³ outdoors. Indoor concentration of TMPD-MIB (2,2,4-trimethyl-1,3-pentanediol monoisobutyrate, Texanol) and TMPD-DIB (2,2,4-trimethyl-1,3-pentanediol diisobutyrate, TXIB), two common plasticizers, were 0.89 and 1.64 µg/m³, respectively. MVOC and plasticizer concentration were correlated ($r = 0.5$; $P < 0.01$). Mold concentration was 360 cfu/m³ indoors and 980 cfu/m³ outdoors. At higher indoor concentrations of total MVOC, nocturnal breathlessness ($P < 0.01$) and doctor-diagnosed asthma ($P < 0.05$) were more common. Moreover, there were positive associations between nocturnal breathlessness and 3-methylfuran ($P < 0.01$), 3-methyl-1-butanol ($P < 0.05$), dimethyldisulfide ($P < 0.01$), 2-heptanone ($P < 0.01$), 1-octen-3-ol ($P < 0.05$), 3-octanone ($P < 0.05$), TMPD-MIB ($P < 0.05$), and TMPD-DIB ($P < 0.01$). TMPD-DIB was positively associated with wheeze ($P < 0.05$), daytime breathlessness ($P < 0.05$), doctor-diagnosed asthma ($P < 0.05$), and current asthma ($P < 0.05$). In conclusion, exposure to MVOC and plasticizers at school may be a risk factor for asthmatic symptoms in children.

J. L. Kim, L. Elfman, Y. Mi, G. Wieslander, G. Smedje, D. Norbäck

Department of Medical Sciences, Occupational and Environmental Medicine, Uppsala University and University Hospital, Uppsala, Sweden

Key words: Asthma; Microbial volatile organic compounds (MVOCs); Plasticizer; Pupil; Respiratory symptoms; School.

Jeong-Lim Kim
Department of Medical Sciences
Occupational and Environmental Medicine
Uppsala University and University Hospital
Uppsala
Sweden
Tel.: +46 18 611 36 47
Fax: +46 18 51 99 78
e-mail: jeong-lim.kim@medsci.uu.se

Received for review 13 January 2006. Accepted for publication 16 September 2006.
© Indoor Air (2006)

Practical Implications

Despite generally good ventilation and lack of visible signs of mold growth, we found an association between respiratory symptoms and indoor MVOC concentration. In addition, we found associations between asthmatic symptoms and two common plasticizers. The highest levels of MVOC, TMPD-MIB, and TMPD-DIB were found in two new buildings, suggesting that material emissions should be better controlled. As MVOC and plasticizers concentrations were positively correlated, while indoor viable molds and bacteria were negatively correlated, it is unclear if indoor MVOC is an indicator of microbial exposure. Further studies focusing on health effects of chemical emissions from indoor plastic materials, including PVC-floor coatings, are needed.

Introduction

Given the rising prevalence of asthma and allergy worldwide (Sennhauser et al., 2005), especially in children, there is a need for evaluating the possible causes and to identify measures to reduce the incidence. There are many different candidate factors for the increase in the incidence asthma and allergy. However,

considering the rapid increase of the prevalence of asthma and allergy in recent decades the potential risk factors can be focused on environmental changes rather than genetic factors. Schools can be regarded as the most important indoor environment for children besides homes. Previous measurements have shown a high concentration of carbon dioxide (CO₂) because of low air exchange rate (Meyer et al., 2004), abundant

airborne particles (Fox et al., 2003; Lee and Chang, 2000; Smedje et al., 1997; Tortolero et al., 2002) and dampness (Taskinen et al., 1997). Possible health effects of school environment with respect to respiratory symptoms, asthma and allergy have been found (Daisy et al., 2003; Munir et al., 1993; Putus et al., 2004; Smedje and Norbäck, 2001; Smedje et al., 1997; Taskinen et al., 1997).

Since the concept of the hygiene hypothesis was introduced (Strachan, 1989), there has been a focus on protective effects by exposure to endotoxins and microbial compounds in early life with respect to asthma and atopy (Braun-Fahrlander, 2003; Douwes et al., 2004; Holt et al., 1999; Martinez and Holt, 1999; von Mutius et al., 2000; Riedler et al., 2001). In contrast, some studies have demonstrated a high prevalence of respiratory symptoms among children living in moisture-damaged or moldy buildings (Bornehag et al., 2005a; Garrett et al., 1998; Mommers et al., 2005; Simoni et al., 2005). Moreover, some studies have reported that microbial exposures in school could be a risk factor for respiratory symptoms, asthma, and allergic triggers (Meklin et al., 2005; Meyer et al., 2004; Putus et al., 2004; Smedje et al., 1996, 1997). Molds and bacteria may produce specific microbial volatile organic compounds (MVOC) (Claeson et al., 2002; Fischer and Dott, 2003; Wessen and Schoeps, 1996) but the specificity of MVOC as an indicator of microbial exposure has been questioned (Pasanen et al., 1998). In addition, it has been claimed that the indoor levels of MVOC are much lower than the sensory irritation thresholds (Korpi et al., 1999; Pasanen et al., 1998). We found only two epidemiological studies on MVOC, both reporting positive associations between indoor MVOC and asthma or allergy (Elke et al., 1999; Smedje et al., 1996). In some countries, MVOC measurements are routinely used by building consultants for investigation of suspected 'sick buildings' (Schleibinger et al., 2005; Wessen and Schoeps, 1996). Recently, associations between childhood asthma and amounts of plastic material in dwellings have been found (Jaakkola et al., 2000; Oie et al., 1997, 1999). Moreover, associations between asthma and allergic symptoms, and polyvinyl chloride (PVC) floor coatings with dampness (Bornehag et al., 2005a) as well as dust concentration of certain phthalate esters has been reported (Bornehag et al., 2004). One common source of these plasticizers is PVC materials (Bornehag et al., 2005b). In damp and alkaline indoor environments, 2-ethyl-1-hexanol may be formed by degradation of diethyl hexyl phthalate (DEHP) in floor materials or water-based glue (Norbäck et al., 2000). In addition, certain bacteria and fungi may produce 2-ethyl-1-hexanol (Nalli et al., 2006). An association between 2-ethyl-1-hexanol and sick building syndrome (Kamijima et al., 2002), nasal symptoms and inflammation (Wieslander et al., 1999) and asthma (Norbäck

et al., 2000) have been reported. TMPD-MIB (2,2,4-trimethyl-1,3-pentanediol monoisobutyrate) and TMPD-DIB (2,2,4-trimethyl-1,3-pentanediol diisobutyrate) are other plasticizers, commonly used in water-based paints (Wieslander et al., 1997), PVC-materials, and other plastic materials such as urethanes wallpaper and artificial leather products (Cain et al., 2005). In a recent review, it was concluded that TMPD-DIB could contribute to indoor odor and irritation (Cain et al., 2005), and it could also be an ambient environmental pollutant (Dsikowitzky et al., 2004). To our knowledge, there are no epidemiological studies available on asthma or allergy in relation to these compounds.

In this study, asthmatic symptoms and self-reported atopic sensitization among primary school pupils were investigated in relation to different types of airborne microbial exposure and plasticizers in the classrooms. The primary objective was to study associations between molds, bacteria, MVOC, formaldehyde, and selected plasticizer compounds in the classrooms in relation to reported current asthma, respiratory symptoms, and allergic triggers among the children. The secondary objective was to study possible associations between indoor microbial and plasticizer exposure and selected building factors in the schools and between microbial exposure and plasticizers.

Material and methods

Study design

All eight primary schools (1–6th form), belonging to Knivsta Municipality, a part of the rural outskirts of Uppsala City, participated in this study. In year 2000, the headmasters were contacted and they all agreed to participate. All pupils in the schools ($N = 1482$) received a questionnaire in Swedish in April–May 2000. Totally, 1014 subjects participated with a response rate of 68%. The questionnaire was sent to the parents by mail, with instructions to answer it in co-operation with the child. The questionnaire contained a set of questions asking about asthma which included doctor-diagnosed asthma, current asthma medication, or asthma attacks during the last 12 months. Current asthma was defined as having either current asthma medication or having had an asthma attack during the last 12 months, a definition previously used in the ECRHS study (Plaschke et al., 2000). Another set of questions asked about airway symptoms related to asthma during the last 12 months, without using the phrase 'asthma'. These symptoms included: (i) wheezing or whistling in the chest, (ii) at least one daytime attack of shortness of breath during exercise or while resting, and (iii) at least one nighttime awakening with attacks of breathlessness or tightness in the chest (Norbäck et al., 1999). Finally the questionnaire contained questions about allergic

triggers, including allergy to pollen and furry pets, which have been used in previous school investigations (Smedje and Norbäck, 2001).

Assessment of exposure

Initial measurements were performed in May and the first week of June 2000, 1–2 weeks after the questionnaire investigation was completed. Measurements were performed in three arbitrarily selected classrooms in each school ($n = 23$), representing different buildings. One of the schools only had two classrooms, of which both were included. Details on constructions, materials, type of ventilation system, cleaning routines, and number of pupils present were noted. The classrooms were inspected for signs of building dampness including visible mold growth on indoor surfaces as well as other signs of building dampness (water leakage, signs of dampness from floor construction such as bubbles under the floor coating, and moldy odor). The room volume (m^3), shelf factor (m/m^3), and textile factor (m^2/m^3) were calculated in each classroom. The shelf factor is defined as the length of open shelves in relation to the room volume and textile factor as the surface area of fabrics in relation to the room volume (Skov et al., 1990). Population density was calculated by dividing number of pupils with classroom volume. In each classroom, room temperature, relative air humidity, air exchange rate (per hour), and concentration of carbon dioxide (CO_2) were measured. The measurements were made during normal activities with closed windows and doors. The average concentration for each school was calculated and used in the statistical models. Outdoor measurements of temperature, relative humidity, and CO_2 were performed during the same period and by the same method as for the indoor measurements.

Temperature, relative air humidity, and CO_2 were measured with Q-TrakTM IAQ Monitor (TSI Inc., ST, Paul, MN, USA), by sampling 1-min average values. The instruments were regularly calibrated by Comfort Control, the Swedish service laboratory for TSI equipment. The air exchange rates in the classrooms were measured with a tracer-gas decay method using acetone as tracer gas (Anundi et al., 1992).

Measurement of indoor microbial and chemical exposure

All hygienic measurements were performed 2–4 weeks after the questionnaire survey was completed (May–June 2000). Airborne microorganisms, volatile organic compounds of possible microbial origin (MVOC), selected plasticizer compounds, and formaldehyde were sampled simultaneously in the classrooms (three samples per school) and outside the building (1 sample/school). Airborne microorganisms were sampled on 25 mm nucleopore filters (pore size $0.4 \mu m$) ($2.0 l/min$;

4 h). The total concentration of airborne molds and bacteria were determined by the CAMNEA method (Palmgren et al., 1986) performed by Pegasus Lab, Uppsala, Sweden. This method is based on acridine orange staining and epifluorescence microscopy. Selected viable molds and bacterial species were determined by incubation on two different media; tryptone glucose agar and malt extract agar (DG18) at $22^\circ C (\pm 1^\circ C)$. The incubation time was 7 days for both media and all microorganisms except for *Streptomyces* sp., where the incubation time was 21 days. The detection limit for viable organisms was 30 colony-forming units per m^3 of air and $11,000/m^3$ for total bacteria and molds. Airborne MVOC were sampled on a charcoal tube (Anasorb 747; SKC Inc., Eighty Four, PA, USA) with a pump with a flow rate of $0.25 l/min$ for 4 h. The tubes were desorbed with 2 ml of methylene chloride and analyzed by selective ion monitoring (SIM) gas chromatography mass spectrometry (GC-MS; Wessen and Schoeps, 1996) by Pegasus Lab. The following compounds were measured: 3-methylfuran, 3-methyl-1-butanol, dimethyldisulfide, 2-hexanone, 2-heptanone, 1-octen-3-ol, 3-octanone, 2-methyl-1-butanol, ethyl-2-methylbutyrate, 2-pentylfuran, isobutylacetate, isobutanol, 1-butanol, 2-pentanol, ethylisobutyrate, 2-ethyl-1-hexanol, TMPD-MIB, and TMPD-DIB. The detection limit was $1 ng/m^3$ for all MVOC and $0.1 \mu g/m^3$ for 2-ethyl-1-hexanol, TMPD-MIB, and TMPD-DIB. The total concentration of the selected MVOC (total MVOC) was defined as mass summation excluding the butanols, 2-ethyl-1-hexanol, TMPD-MIB, and TMPD-DIB. Indoor concentrations of formaldehyde were measured with glass fiber filters impregnated with 2,4-dinitro-phenylhydrazine (Andersson et al., 1981) with an air sampling flow of $0.2 l/min$ during 6 h. The filters were analyzed by liquid chromatography at the Department of Occupational and Environmental Medicine, Örebro, Sweden. The detection limit for formaldehyde was $6 \mu g/m^3$.

Statistical methods

Arithmetic mean value with standard deviation and min.–max. value were calculated for indoor climate and ventilation. Relationships between the microbial exposure and asthma and allergic triggers were evaluated by multiple logistic regression analysis, adjusting for age and gender. For the statistical calculations, exposure levels below the detection limit were assigned a value of half of the detection limit. Mean exposure levels were calculated for each school and used in the regression models. Difference in asthma and allergic triggers between boys and girls were analyzed by chi-square test. Correlations between total MVOC and climate conditions (air exchange rate, temperature, air humidity and CO_2), shelf and textile factor, microorganisms, and other selected plasticizers were performed

by Kendall's tau-beta rank correlation test. All statistical calculations were performed with Statistical Program for Social Sciences (SPSS Inc., Chicago, IL, USA) for Windows, version 12.0.1. Interaction terms in logistic regression analysis were tested statistically, using the STATA program (Stata Corporation, College Station, TX, USA). In all statistical analyses, two-tailed tests and a 5% level of significance were applied.

Results

Asthma and allergic triggers by questionnaire

In total, 1482 pupils received the questionnaire, of which 1014 (68%) were answered by parents in co-operation with the child. The median age was 9 years (range 5–14, s.d. 2.0) and 51% were girls. The results of the questionnaire are shown in Table 1. Generally, boys reported more symptoms than girls. The most common current airway symptom was wheeze or whistling in the chest for both boys and girls. When asking about symptoms with the phrase 'asthma', girls reported less ever had doctor-diagnosed asthma ($P < 0.01$), current asthma medication ($P < 0.01$), and current asthma ($P < 0.05$). Moreover, the girls reported less dog allergy ($P < 0.05$) and pollen allergy ($P < 0.05$) than boys.

The school environment

The mean age of the school buildings was 46 years (range 1–103). Seven schools had mechanical ventilation, with supply and exhaust air systems and without air humidification, while one had natural ventilation.

Table 1 Reports on current airway symptoms, asthma and allergic triggers among pupils in Knivsta

	Boys (%) (<i>N</i> = 492)	Girls (%) (<i>N</i> = 522)	Total (%) (<i>N</i> = 1014)
Questions on current airway symptoms			
Wheeze or whistling in the chest	8.6	7.1	7.8
Daytime attacks of breathlessness	4.4	4.6	4.5
Nocturnal attacks of breathlessness	2.3	1.7	2.0
At least one airway symptom ^a	10.5	8.7	9.5
Questions on asthma			
Ever had doctor-diagnosed asthma	10.2	5.4**	7.7
Current asthma medication	6.3	3.9**	5.3
Current asthma attacks	3.6	2.6	3.1
Current asthma ^b	7.9	4.1*	5.9
Questions on allergic triggers			
Cat allergy	8.0	5.6	6.8
Dog allergy	6.1	3.5*	4.8
Pollen allergy	10.7	7.0*	8.8
Pollen or furry pet allergy	14.0	10.4	12.2

^aWheeze or whistling in the chest, attacks of daytime breathlessness at rest or at exercise, or attacks of nocturnal breathlessness during the last 12 months.

^bCurrent asthma medication or asthma attacks during the last 12 months.

Calculated by chi-square test for fourfold contingency tables.

* $P < 0.05$, ** $P < 0.01$.

Totally, 14 of the classrooms had displacement ventilation, while eight classrooms had mixing supply-exhaust ventilation systems. Five of the school buildings (63%) were made of wood, while the other three were of concrete constructions. All classrooms had hard floor coverings made either from PVC or linoleum. None of the classrooms had visible signs of dampness, mold growth, or moldy odor. We had no possibilities to investigate the hidden dampness inside the construction. Such investigations generally need to make holes and remove bits of materials from the building. There was no indication of hidden dampness and none of the schools had been investigated by building consultants for dampness problem. Most of the classrooms had daily floor cleaning, either by dry mopping or wet mopping, and desks were wiped 1–5 times a week.

Measurements of indoor and outdoor climate and ventilation are given in Table 2. The average room temperature of the classrooms was 23.2°C, whereas the outdoor temperature was 16°C. The mean relative air humidity was 41% indoors, while it was 57% outdoors. The mean air exchange rate in the classrooms was 4.5 ac/h and the mean CO₂-values were below 1000 ppm in all classrooms. The mean population density was 0.112 (students/m³).

Microorganisms, MVOC, and selected plasticizer compounds indoor and outdoor

Table 3 shows the mean concentration of indoor and outdoor microorganisms, MVOC, formaldehyde, and selected plasticizer compounds. Numerically, the concentration of viable bacteria, viable molds, and total molds were higher outdoors, while the mean concentration of total bacteria was higher indoors. Only viable molds had statistically significant difference between indoor and outdoor ($P < 0.05$). The concentration of most of the MVOC was 3–5 times higher indoors when compared with outdoors. The mean of total MVOC was 423 ng/m³ in the classrooms while 123 ng/m³ in outdoor air and the difference between

Table 2 Indoor/outdoor climate and ventilation in Knivsta schools (May–June)

	<i>N</i>	Mean (s.d.)	Min.–max. value
Room temperature, indoors (°C)	23	23.2 (1.5)	21.0–26.6
Outdoor temperature (°C)	8	16.3 (4.3)	12.0–22.9
Indoor relative air humidity (%)	23	41 (6.9)	27–51
Outdoor relative humidity (%)	8	57 (15.1)	36–77
Air exchange rate (per hour)	23	4.5 (2.0)	0.5–8.1
Room volume (m ³)	23	202 (87.1)	82–470
No. of students/classroom	23	20 (6.8)	8–43
Carbon dioxide (ppm)	23	815 (216)	540–1275
Textile factor (textiles/room vol.)	23	0.074 (0.083)	0.012–0.362
Shelf factor (shelves/room vol.)	23	0.104 (0.063)	0–0.218

Mean (s.d.) = arithmetic mean with standard deviation; *N* = 23 classrooms or eight schools.

Table 3 Average indoor and outdoor concentration of microorganisms, microbial volatile organic compound (MVOC) and specific plasticizer compounds in Knivsta schools

	Classrooms (N)	Indoor	Schools (N)	Outdoor
Microorganisms (10 ³ /m ³)				
Viable bacteria	23	1.77 (1.52)	8	2.20 (4.51)
Total bacteria	23	17.0 (17.0)	8	12.6 (13.7)
Viable molds	23	0.36 (0.31)	8	0.98 (0.65)
Total molds	23	14.8 (15.4)	8	28.4 (39.8)
MVOC (ng/m ³)				
3-Methylfuran	23	19 (<1–160)	8	14 (<1–41)
3-Methyl-1-butanol	23	95 (<1–560)	7	<1
Dimethyldisulfide	23	46 (<1–710)	8	41 (<1–260)
2-Hexanone	23	39 (7–140)	8	14 (1–44)
2-Heptanone	23	121 (32–750)	8	45 (8–170)
1-Octen-3-ol	23	53 (<1–210)	8	5 (<1–16)
3-Octanone	23	11 (<1–24)	8	5 (<1–16)
2-Methyl-1-butanol	21	14 (<1–37)	8	3 (<1–24)
Ethyl-2-methylbutyrate	23	8 (<1–89)	8	2 (<1–15)
2-Pentylfuran	23	45 (13–180)	8	10 (1–31)
Isobutylacetate	23	271 (<1–2680)	8	<1
Isobutanol	23	355 (94–1380)	8	60 (15–210)
1-Butanol	23	1291 (440–3520)	8	242 (40–1340)
2-Pentanol	23	41 (<1–320)	8	<1
Ethylisobutyrate	23	<1	8	<1
Total MVOC ^a	23	423 (66–2270)	8	123 (8–530)
Other specific compounds (μg/m ³)				
Formaldehyde	23	7.13 (3–16)		NA
2-Ethyl-1-hexanol	22	0.83 (0.25–1.98)	8	0.19 (0.08–0.27)
TMPD-MIB	23	0.89 (0.07–4.41)	8	0.17 (0.10–0.26)
TMPD-DIB	23	1.64 (0.68–3.42)	8	0.41 (0.24–0.75)

Values are arithmetic mean (s.d.) or (range).

^aSum of 3-methylfuran, 3-methyl-1-butanol, dimethyldisulfide, 2-hexanone, 2-heptanone, 1-octen-3-ol, 3-octanone, 2-methyl-1-butanol, ethyl-2-methylbutyrate, 2-pentylfuran, isobutylacetate, 2-pentanol and ethylisobutyrate.

indoor and outdoor was significant ($P < 0.05$). The mean indoor concentration of plasticizer compounds was higher than outdoor. Viable bacteria, viable molds, 3-methylfuran, 2-hexanone, 2-heptanone, 2-pentylfuran, isobutanol, 1-butanol, 2-ethyl-1-hexanol, TMPD-MIB, and TMPD-DIB were above the detection limit in all samples. Total bacteria and total molds were below the detection limit in 13 of 23 indoor samples (57%). 3-Methyl-1-butanol, dimethyldisulfide, 1-octen-3-ol, 2-octanone, 2-methyl-1-butanol, ethyl-2-methylbutyrate, isobutylacetate, 2-pentanol, and formaldehyde were below the detection limit in 13, 10, 1, 1, 13, 17, 13, 17, and 7 indoor samples, respectively. Ethylisobutyrate was not detected in any sample. Table 4 shows the type of viable microbial species identified in indoor and outdoor air. *Cladosporium* sp., *Sterile mycelia* and the gram-negative bacteria *Pseudomonas* sp. were most common, both indoors and outdoors. Other species were more uncommon and found mostly indoors.

Association between microorganisms, MVOC, formaldehyde, and selected plasticizer compounds, and asthma and allergic triggers

Distribution of mean exposure and symptom prevalence in the eight schools is given in Table 5. School

Table 4 Types of viable microorganisms in air samples from classrooms and from outdoors

	Indoor prevalence (%)		Outdoor prevalence (%)
	Classrooms (N = 23)	School ^a (N = 8)	School ^a (N = 8)
<i>Cladosporium</i> sp.	87	100	100
<i>Sterile mycelia</i>	48	100	75
<i>Pseudomonas</i> sp.	57	75	38
Yeast	30	50	13
<i>Aurobasidium</i> sp.	13	38	25
<i>Penicillium</i> sp.	17	38	13
<i>Streptomyces</i> sp.	17	38	25
<i>Phoma</i> sp.	9	25	0
<i>Bacillus</i> sp.	4	13	13
<i>Crysonilia</i> sp.	4	13	13
<i>Pezizia</i> sp.	4	13	0
<i>Aspergillus</i> sp.	0	0	0

^aPresent in at least one air sample from the school.

number 5 had a major reconstruction in 1999 and school number 6 was constructed in 1999. These new schools with new building materials had the highest levels of total MVOC, TMPD-MIB, and TMPD-DIB. In addition, pupils in these schools reported the highest prevalence of asthma and some respiratory symptoms. In the multiple logistic regression model, when adjusting for age and gender, both concentration of total bacteria ($P < 0.05$) and concentration of total molds ($P < 0.05$) were negatively associated with at least one respiratory symptom (Table 6). In addition, concentration of total molds had a negative association with wheeze and concentration of viable bacteria had a negative association with doctor-diagnosed asthma ($P < 0.05$). Furthermore, both concentration of viable ($P < 0.01$) and total bacteria ($P < 0.05$) had an inverse (negative) association with nocturnal breathlessness. On the contrary, most of specific MVOC showed a significant positive relationship with nocturnal breathlessness. Moreover, 3-octanone was positively related with wheeze ($P < 0.05$) and at least one respiratory symptom ($P < 0.05$). 2-Heptanone and total MVOC were positively related with doctor-diagnosed asthma ($P < 0.05$). Among selected plasticizer compounds, TMPD-DIB presented significant positive associations with all different respiratory symptoms, doctor-diagnosed asthma, and current asthma (OR = 1.99, 95% CI 1.05–3.77 for a change of 1 μg/m³; $P < 0.05$). TMPD-MIB was associated with only nocturnal breathlessness. No association was found between a history of atopic sensitization and any MVOC, plasticizer compounds, or formaldehyde in indoor air.

Correlation between microorganisms, MVOC, formaldehyde, and selected plasticizer compounds and climate, textile, and shelf factor

Table 7 shows correlations between different indoor factors and microorganisms, MVOC, formaldehyde,

Table 5 Mean exposures, building age and symptom prevalence in the year 2000 among the pupils in eight schools

School	Year ^a	Pupils ^b (n)	Air exchange	Bacteria (10 ³ /m ³)		Molds (10 ³ /m ³)		Total MVOC (ng/m ³)	Formaldehyde (μg/m ³)	2-Ethyl-1-hexanol (μg/m ³)	TMPD-MIB (μg/m ³)	TMPD-DIB (μg/m ³)	Wheeze	Breathlessness (%)		Doctor-diagnosed asthma (%)
			Rate (per hour)	Viable	Total	Viable	Total							Daytime	Nocturnal	
1	1955	185	3.87	740	19,800	430	14,300	0.19	4.0	0.75	0.46	2.02	10.3	8.8	1.7	9.2
2	1983	272	4.07	2480	23,800	880	38,000	0.11	8.3	0.58	0.29	1.12	4.8	3.4	1.1	6.3
3	1897	55	5.57	4570	17,500	440	10,500	0.08	5.8	0.35	0.35	1.12	7.3	1.9	0	3.6
4	1990	126	4.93	2170	16,500	320	7300	0.21	5.7	1.03	0.89	1.35	10.3	3.3	1.6	4.8
5	1999	164	5.50	590	5500	140	5500	1.01	11.0	0.78	3.28	2.12	10.4	5.6	4.3	10.4
6	1999	17	3.75	460	8500	180	8000	1.38	7.0	1.61	0.92	2.64	11.8	5.9	18.8	18.8
7	1977	106	2.50	1410	19,200	260	2300	0.52	10.3	1.28	0.65	1.61	5.8	1.9	0	7.5
8	1997	89	5.87	620	22,300	170	5500	0.20	7.8	0.76	0.33	1.47	5.7	3.4	2.2	9.0

^aConstruction year or year of recent major reconstruction.^bNumber of participating pupils.**Table 6** Association between different exposures in the school and respiratory symptoms

Type of indoor exposure	Wheeze	Daytime breathlessness	Nocturnal breathlessness	Doctor-diagnosed asthma
Viable bacteria ^a	0.99 (0.96–1.01)	0.97 (0.94–1.01)	0.92 (0.87–0.98)**	0.97 (0.94–1.00)*
Total bacteria ^b	0.97 (0.93–1.00)	0.96 (0.91–1.01)	0.92 (0.87–0.98)*	0.97 (0.95–1.21)
Viable molds ^a	0.93 (0.85–1.01)	0.94 (0.84–1.05)	0.84 (0.69–1.03)	0.94 (0.86–1.02)
Total molds ^b	0.98 (0.96–1.00)*	0.98 (0.95–1.01)	0.96 (0.92–1.00)	0.99 (0.97–1.01)
MVOC				
3-Methylfuran ^c	1.07 (0.90–1.28)	1.07 (0.81–1.40)	1.31 (1.08–1.59)**	1.16 (0.98–1.37)
3-Methyl-1-butanol ^c	1.01 (0.99–1.02)	1.01 (0.99–1.03)	1.03 (1.00–1.05)*	1.01 (1.00–1.03)
Dimethyldisulfide ^c	1.02 (0.97–1.06)	1.02 (0.96–1.08)	1.07 (1.02–1.11)**	1.04 (1.00–1.08)
2-Hexanone ^c	1.05 (0.94–1.18)	1.03 (0.88–1.21)	1.17 (0.98–1.40)	1.09 (0.98–1.22)
2-Heptanone ^c	1.02 (0.99–1.05)	1.02 (0.98–1.07)	1.06 (1.02–1.10)**	1.03 (1.00–1.06)*
1-Octen-3-ol ^c	1.05 (0.96–1.14)	1.04 (0.92–1.17)	1.18 (1.03–1.35)*	1.08 (0.99–1.18)
3-Octanone ^c	1.61 (1.02–2.56)*	1.73 (0.92–3.27)	3.04 (1.19–7.81)*	1.57 (0.98–2.52)
2-Methyl-1-butanol ^c	1.01 (0.83–1.24)	1.10 (0.83–1.45)	1.54 (1.22–1.95)***	1.25 (1.05–1.50)*
Ethyl-2-methylbutyrate ^c	0.95 (0.75–1.20)	0.78 (0.53–1.15)	0.85 (0.51–1.42)	1.03 (0.83–1.28)
2-Pentylfuran ^c	1.07 (0.97–1.18)	1.06 (0.93–1.22)	1.21 (1.04–1.41)*	1.10 (1.00–1.22)
2-Pentanol ^c	1.01 (0.99–1.04)	1.02 (0.99–1.06)	1.04 (1.00–1.08)	1.02 (0.99–1.05)
Isobutylacetate ^d	1.20 (0.84–1.71)	1.41 (0.88–2.28)	1.77 (1.00–3.14)*	1.32 (0.92–1.89)
Isobutanol ^d	2.74 (0.71–10.57)	2.05 (0.30–13.87)	7.23 (0.60–87.16)	1.43 (0.35–5.87)
1-Butanol ^d	1.26 (0.76–2.10)	1.34 (0.67–2.83)	3.41 (1.38–8.46)**	1.67 (0.99–2.80)
Total MVOC ^d	1.46 (0.77–2.79)	1.82 (0.74–4.47)	5.25 (1.82–15.18)**	2.07 (1.09–3.93)*
Other specific compounds				
Formaldehyde ^d	0.96 (0.87–1.05)	0.96 (0.85–1.08)	1.03 (0.86–1.24)	1.02 (0.93–1.13)
2-Ethyl-1-hexanol ^d	1.48 (0.62–3.53)	0.96 (0.27–3.35)	3.16 (0.72–13.89)	1.70 (0.70–4.11)
TMPD-MIB ^d	1.15 (0.94–1.42)	1.24 (0.94–1.65)	1.41 (1.00–1.98)*	1.17 (0.94–1.44)
TMPD-DIB ^d	1.85 (1.08–3.17)*	2.39 (1.15–4.96)*	5.71 (2.04–16.02)**	1.97 (1.14–3.42)*

Odds ratio expressed as change of coefficient per ^a10²/m³, ^b10³/m³, ^c10 ng/m³ and ^d1 μg/m³.**P* < 0.05, ***P* < 0.01, ****P* < 0.001. Adjustment for age and gender by multiple logistic regression.**Table 7** Correlation between selected indoor variables

	Total MVOC (<i>R</i>)	Formaldehyde (<i>R</i>)	2-Ethyl-1-hexanol (<i>R</i>)	TMPD-MIB (<i>R</i>)	TMPD-DIB (<i>R</i>)
Temperature	0.028	0.092	0.341*	0.056	0.107
Relative humidity	0.036	0.265	0.242	0.369*	0.124
Air exchange rate	0.120	−0.314*	0.268	0.187	0.207
Population density	0.286	0.071	0.118	−0.472**	−0.325*
CO ₂	0.257	0.315*	0.084	0.060	0.080
Viable bacteria	−0.463**	0.029	−0.338*	0.227	0.247
Viable mold	−0.441**	0.080	0.242	0.148	0.216
Total MVOC	—	0.259	0.534**	0.477**	0.528**

R = correlation coefficient by Kendall's tau-beta rank correlation test.**P* < 0.05, ***P* < 0.01.

and plasticizer compounds. There was no association between textile factor, shelf factor, total bacteria, or total molds and any indoor exposure. Viable bacteria and viable molds were negatively correlated with the total MVOC. Formaldehyde was positively correlated with CO₂ and negatively correlated with air exchange. Concentration of 2-ethyl-1-hexanol was positively associated with room temperature and the total MVOC and negatively correlated with viable bacteria. TMPD-MIB was positively correlated with relative air humidity and total MVOC, and negatively associated with population density. TMPD-DIB was positively associated with the total MVOC and negatively associated with population density. Moreover, the plasticizers were positively correlated. TMPD-DIB was correlated with both 2-ethyl-1-hexanol (correlation coefficient = 0.54; $P < 0.001$) and TMPD-MIB (correlation coefficient = 0.45; $P = 0.003$), while 2-ethyl-1-hexanol and TMPD-MIB were not significantly correlated (correlation coefficient = 0.25; NS). Formaldehyde was not correlated with any plasticizer compound (data not shown in table). Moreover, we evaluated possible interaction between ventilation and exposure. We made a stratified analysis with respect to low and high ventilation (air exchange rate). The stratification was based on median of air exchange rate (four schools were below and four schools were above 4.4 ac/h). There was no significant difference in exposure levels in classrooms of total MVOC, TMPD-MIB, and TMPD-DIB between the two groups. The associations between exposure, wheeze, and nocturnal breathlessness were significant only in the low ventilation group. Finally, we performed an interaction analysis using the total material. None of the interaction terms between air exchange rate and exposures was statistically significant. There was a tendency for interaction between air exchange and TMPD-DIB, with respect to nocturnal attacks of breathlessness (interaction term $P = 0.08$).

Discussion

This study found that the indoor concentration of MVOC as well as the plasticizers TMPD-MIB and TMPD-DIB in schools were associated with increased prevalence of asthmatic symptoms in pupils. The effect was most consistent for TMPD-DIB, which was related to both asthma and various respiratory symptoms. In addition, we found an association between indoor levels of MVOC and both plasticizers. The indoor concentration of MVOC was 3–5 times higher when compared with outdoor levels, indicating significant indoor sources despite lack of visible signs of dampness or mold growth. In contrast, the outdoor levels of molds were higher. This suggests that MVOC and plasticizers may have health risks, even in schools with good

ventilation. Moreover, it is unclear if MVOC is an indicator of microbial exposure.

In this study, all primary schools in one municipality of Uppsala were included for both the questionnaire survey and environmental measurements. The response rate from the questionnaire study was 68% and there was no significant difference in symptom reporting when comparing answers given by father or mother. Moreover, the proportion of girls was 51%, indicating no gender selection. For the environmental hygienic measurement, the classrooms were arbitrarily chosen in all schools and all sampling was performed by the same professional environmental engineers 2–4 weeks after the questionnaire survey was completed. As total bacteria and molds were below the detection limit in half of samples, conclusions on these exposures are less reliable than association on viable bacteria. Only few MVOC values were below the detection limit and none of the plasticizers was below the detection limit. A number of statistical tests were made and consequently some associations could be because of mass significance. However, the results were consistent with regard to different MVOC and respiratory symptoms, especially nocturnal breathlessness, and between TMPD-DIB and both asthma and various respiratory symptoms. Moreover, the patterns of association were consistent and more pronounced in the schools with lower ventilation. Therefore, our study can be considered to have good internal validity and our conclusions should not be seriously affected by selection or information bias or mass significance.

The higher prevalence of respiratory symptoms, asthma, and allergic triggers in boys is consistent with previous studies (Forsberg et al., 1997; Morgan and Martinez, 1992; Rönmark et al., 1998). Moreover, the prevalence of self-reported asthma and allergic triggers was similar to another school study performed in 1993 in the same area of Sweden among 1347 pupils with the mean age of 10.3 years. In our study, 7.7% had doctor-diagnosed asthma, 8.8% pollen allergy, and 7.3% pet allergy. The former study reported 6.6% doctor-diagnosed asthma, 9.8% pollen allergy, and 8.2% pet allergy (Smedje and Norbäck, 2001).

We found an association between MVOC exposure at schools and both asthma and asthmatic symptoms, mainly for nocturnal breathlessness. The compound 3-octanone was significantly associated with wheeze, and 2-heptanone and 2-methyl-1-butanol were associated with doctor-diagnosed asthma as well. Totally, 10 of 14 MVOCs were associated with nocturnal attacks of breathlessness, of which five with significant P -values below 0.01. Associations between indoor VOC in dwellings and attacks of nocturnal breathlessness have previously been reported (Norbäck et al., 1995). We have found only two previous epidemiological studies on MVOC in relation to asthma and asthmatic symptoms, both reporting significant positive

associations between MVOC and asthma (Elke et al., 1999; Smedje et al., 1996). In addition, one experimental exposure chamber study showed that 3-methylfuran, one common MVOC, can cause increase in inflammatory biomarkers in nasal lavage and decreased forced vital capacity of the lungs (Wålinder et al., 2005). Moreover, one atopic subject with previous occupational exposure to molds got an acute airway obstruction and delayed pulmonary reactions with influenza like symptoms after being exposed to 3-methylfuran (Wålinder et al., 1998). This indicates that delayed respiratory reactions to MVOCs may occur, but the concentration of 3-methylfuran in the exposure chamber study was much higher than in our schools. Generally, the concentration of MVOC in our study was similar to another Swedish school study (Smedje et al., 1996) using the same analytical laboratory as we have used. In contrast, a German study reported higher levels of MVOC. However, that study was performed in dwellings and used a less specific analytical method (dual-column gas chromatography with flame ionization detection) (Elke et al., 1999), while we used GC-MS with SIM (Wessen and Schoeps, 1996). Despite no visible signs of dampness and molds in any classroom, there were large variations of MVOC concentrations which were not related to air exchange rate, population density, shelf or textile factor, but associated with air concentrations of plasticizers. Laboratory measurements of material samples have shown some increase of MVOC when the material is moldy, but emission of MVOC may also occur from reference materials (Pasanen et al., 1998). Therefore, it is unclear as to what extent MVOC is a specific indicator of indoor microbial exposure. In order to clarify the usefulness of MVOC as an indicator of hidden mold growth, further field studies are needed.

In this study, outdoor concentration of viable molds was higher than indoors, whereas indoor and outdoor concentration of bacteria were the same. Other studies have shown that pupils are a significant indoor source of bacterial components, such as muramic acid (Fox et al., 2003) but we found no association between population density and indoor bacteria. Both indoor and outdoor microbial exposure may be influenced by season. The warmer season results in more outdoor microbial activity. Thus, there could be an influence on indoor levels of microorganisms and MVOC from outdoor sources. Our microbial measurements were performed in May–June. We have published data on ventilation and indoor air relative humidity comparing May and December in these particular schools (Kim et al., 2005). Air exchange rate was slightly higher (20%), relative humidity was lower (10%), and room temperature was 2°C lower in winter. As indoor levels of MVOC were higher than outdoor levels even in May (warmer season), outdoor influence on indoor MVOC levels should be less than that for microorganisms, but

we do not have actual measured data from the schools supporting this assumption.

Surprisingly, we found negative associations between indoor microbial exposure and asthmatic symptoms. This is in contrast with a previous school study from the same area, reporting positive associations between indoor viable molds and bacteria measured in spring, and current asthma (Smedje et al., 1997). A protective effect of bacterial exposure on asthma and atopic sensitization in early childhood, especially from gram-negative bacteria, has been suggested (Holt et al., 1999). We measured one type of gram-negative bacteria, *Pseudomonas* sp., and it was commonly detected both indoors and outdoors. Therefore, our results could be interpreted as a protective effect of bacterial exposure, as previously reported from Chinese schools (Norbäck et al., 2002). However, as we found negative associations for both molds and bacteria, with respect to asthmatic symptoms, it is more likely that the findings arose out of differences in window or door opening habits. When we performed our measurements, the teachers were asked to keep the windows closed. Increased window opening during normal conditions would lead to better ventilation, but more accumulation of outdoor microorganisms in the classroom. Thus, differences in airing habits may explain the negative associations between microbial exposure and asthmatic symptoms.

Besides the association with MVOC, we found positive associations between TMPD-MIB and TMPD-DIB, two common plasticizers, and asthma and asthmatic symptoms. The highest levels of MVOC, TMPD-MIB, and TMPD-DIB were found in two schools, which were newly constructed/reconstructed, suggesting that one source could be emissions from new building materials. We have found no other epidemiological studies on associations between these compounds and asthma. TMPD-MIB was associated with only nocturnal attacks of breathlessness, while TMPD-DIB was associated with both asthma and various types of asthmatic symptoms. However, there was no association between 2-ethyl-1-hexanol and asthma or respiratory symptoms. The levels of 2-ethyl-1-hexanol were low ($0.2\text{--}2.0\text{ }\mu\text{g}/\text{m}^3$) in the classrooms and were equal to what is normally found indoors. Levels above $5\text{--}10\text{ }\mu\text{g}/\text{m}^3$ are found in buildings with floor dampness, causing excessive emission of 2-ethyl-1-hexanol formed by alkaline degradation of DEHP in PVC floor materials (Wieslander et al., 1999). TMPD-MIB is found in water-based paints (Sparks et al., 1999; Wieslander et al., 1997), and levels in the range of $30\text{--}100\text{ }\mu\text{g}/\text{m}^3$ are generally found in recently painted indoor environments (Sparks et al., 1999). Our indoor levels ($0.07\text{--}4.4\text{ }\mu\text{g}/\text{m}^3$) were much lower, as none of the schools had been painted during last year. TMPD-DIB is mainly emitted from PVC-materials but is also used as plasticizer in other

polymer products (Cain et al., 2005). Our levels ($0.7\text{--}3.4\text{ }\mu\text{g}/\text{m}^3$) were similar to what is normally found in dwellings and were much lower than the levels detected in recently painted dwellings (Wieslander et al., 1997). One experimental study concluded that TMPD-DIB can contribute to odor at ppb levels, but irritation occurs only at higher levels (Cain et al., 2005). On the other hand, it has been demonstrated that TMPD-DIB is found in higher levels in dwellings with health problems ('sick houses') when compared with normal houses (Kostiainen, 1995). Moreover, an association between TMPD-DIB-levels in dwellings and clinical signs of eosinophilic inflammation has been demonstrated (Norbäck et al., 1993). As TMPD-DIB and phthalate esters are both used as plasticizers in PVC-materials, it is likely that these compounds are associated with indoor environment. We did not measure

phthalates in our study and other studies on health effects of phthalate plasticizers (Bornehag et al., 2004; Shea et al., 2003) usually have not measured TMPD-DIB. Thus, TMPD-DIB levels could be a proxy variable for phthalate exposure, and vice versa, and in future studies exposure to different types of plasticizer compounds should be monitored.

To conclude, exposure to MVOC, TMPD-MIB, and TMPD-DIB in the school environment can be associated with asthmatic symptoms in children. The associations were stronger in schools with lower air exchange rate indicating possible interaction between exposure and ventilation with respect to symptoms. It is unclear as to what extent MVOC is a specific marker of microbial exposure. Finally, as there are not many studies dealing with MVOC or plasticizers as health risks in school environment, further studies are needed.

References

- Andersson, K., Hallgren, G., Levin, J.O. and Nilsson, C.A. (1981) Chemosorption sampling and analysis of formaldehyde in air; influence on recovery during simultaneous sampling of formaldehyde, phenol, furfural alcohol, *Scand. J. Work Environ. Health*, **7**, 282–289.
- Anundi, H., Norbäck, D., Kinigalakis, G. and Johanson, G. (1992) Simplified measurement of air-exchange rate using acetone as tracer gas. *Proceedings from 41 Nordic Meeting of Work Environment*, Sept 2–4, 1992 (in Swedish), Reykjavik, Statens Arbetsmiljöstyrelse, 227–228.
- Bornehag, C.G., Sundell, J., Weschler, C.J., Sigsgaard, T., Lundgren, B., Hasselgren, M. and Hägerhed-Engman, L. (2004) The association between asthma and allergic symptoms in children and phthalates in house dust: a nested case-control study, *Environ. Health Perspect.*, **112**, 1393–1397.
- Bornehag, C.G., Sundell, J., Hagerhed-Engman, L., Sigsgaard, T., Janson, S., Aberg, N. and the DBH Study Group. (2005a) 'Dampness' at home and its association with airway, nose, and skin symptoms among 10,851 preschool children in Sweden: a cross-sectional study, *Indoor Air*, **15** (Suppl. 10), 48–55.
- Bornehag, C.G., Lundgren, B., Weschler, C.J., Sigsgaard, T., Hagerhed-Engman, L. and Sundell, J. (2005b) Phthalates in indoor dust and their association with building characteristics, *Environ. Health Perspect.*, **113**, 1399–1404.
- Braun-Fahrlander, C. (2003) Environmental exposure to endotoxin and other microbial products and the decreased risk of childhood atopy: evaluating developments since April 2002, *Curr. Opin. Allergy Clin. Immunol.*, **3**, 325–329.
- Cain, W.S., de Wijk, R.A., Jalowayski, A.A., Pilla Caminha, G. and Schmidt, R. (2005) Odor and chemesthesis from brief exposures to TXIB, *Indoor Air*, **15**, 445–457.
- Claeson, A.S., Levin, J.O., Blomquist, G. and Sunesson, A.L. (2002) Volatile metabolites from microorganisms grown on humid building materials and synthetic media, *J. Environ. Monit.*, **4**, 667–672.
- Daisy, J.M., Angeli, W.J. and Apte, M.G. (2003) Indoor air quality, ventilation and health symptoms in schools: an analysis of existing information, *Indoor Air*, **13**, 53–64.
- Douwes, J., Le Gros, G., Gibson, P. and Pearce, N. (2004) Can bacterial endotoxin exposure reverse atopy and atopic disease? *J. Allergy Clin. Immunol.*, **115**, 1325–1326.
- Dsikowitzky, L., Schwarzbauer, J. and Litke, R. (2004) The anthropogenic contribution to the organic load of the Lippe River (Germany). Part II: quantification of specific organic contaminants, *Chemosphere*, **57**, 1289–1300.
- Elke, K., Begerow, J., Oppermann, H., Kramer, U., Jermann, E. and Dunemann, L. (1999) Determination of selected microbial volatile organic compounds by diffusive sampling and dual-column capillary GC-FID – a new feasible approach for the detection of an exposure to indoor mould fungi?, *J. Environ. Monit.*, **1**, 445–452.
- Fischer, G. and Dott, W. (2003) Relevance of airborne fungi and their secondary metabolites for environmental, occupational and indoor hygiene, *Arch. Microbiol.*, **179**, 75–82.
- Forsberg, B., Pekkanen, J., Clench-Aas, J., Mårtensson, M.-B., Stjernberg, N., Bartonova, A., Timonen, K.L. and Skerfving, M.S. (1997) Childhood asthma in four regions in Scandinavia: risk factors and avoidance effects, *Int. J. Epidemiol.*, **26**, 610–619.
- Fox, A., Harley, W., Feigley, C., Salzberg, D., Sebastian, A. and Larsson, L. (2003) Increased levels of bacterial markers and CO₂ in occupied school rooms, *J. Environ. Monit.*, **5**, 246–252.
- Garrett, M.H., Rayment, P.R., Hooper, M.A., Abramson, M.J. and Hooper, B.M. (1998) Indoor airborne fungal spores, house dampness and associations with environmental factors and respiratory health in children, *Clin. Exp. Allergy*, **28**, 459–467.
- Holt, P.G., Macaubas, C., Prescott, S.L. and Sly, P.D. (1999) Microbial stimulation as an aetiological factor in atopic disease, *Allergy*, **54**, 12–16.
- Jaakkola, J.K., Verkasalo, P.K. and Jaakkola, N. (2000) Plastic wall materials in the home and respiratory health in young children, *Am. J. Public Health*, **90**, 797–799.
- Kamijima, M., Sakai, K., Shibata, E., Yamada, T., Itoharu, S., Ohno, H., Hayakawa, R., Sugiura, M., Yamaki, K. and Takeuchi, Y. (2002) 2-ethyl-1-hexanol in indoor air as a possible cause of sick building symptoms, *J. Occup. Health*, **44**, 186–191.
- Kim, J.L., Elfman, L., Mi, Y., Johansson, M., Smedje, G. and Norbäck, D. (2005) Current asthma and respiratory symptoms among pupils in relation to dietary factors and allergens in the school environment, *Indoor Air*, **15**, 170–182.
- Korpi, A., Kasanen, J.P., Alarie, Y., Kosma, V.M. and Pasanen, A.L. (1999) Sensory irritating potency of some microbial volatile organic compounds (MVOCs) and a mixture of five MVOCs, *Arch. Environ. Health*, **54**, 347–352.

- Kostiainen, R. (1995) Volatile organic compounds in the indoor air of normal and sick houses, *Atmos. Environ.*, **29**, 693–702.
- Lee, S.C. and Chang, M. (2000) Indoor and outdoor air quality investigation at schools in Hong Kong, *Chemosphere*, **41**, 109–113.
- Martinez, F.D. and Holt, P.G. (1999) Role of microbial burden in aetiology of allergy and asthma, *Lancet*, **354** (Suppl. 2), 12–15.
- Meklin, T., Potus, T., Pekkanen, J., Hyvärinen, A., Hirvonen, M.R. and Nevalainen, A. (2005) Effects of moisture-damage repairs on microbial exposure and symptoms in schoolchildren, *Indoor Air*, **15** (Suppl. 10), 40–47.
- Meyer, H.W., Würtz, H., Suadicani, P., Valbjørn, O., Sigsgaard, T. and Gyntelberg, F. (2004) Molds in floor dust and building-related symptoms in adolescent school children, *Indoor Air*, **14**, 65–72.
- Mommers, M., Jongmans-Liedekerken, A.W., Derkx, R., Dott, W., Mertens, P., van Schayck, C.P., Steup, A., Swaen, G.M., Ziemer, B. and Weishoff-Houben, M. (2005) Indoor environment and respiratory symptoms in children living in the Dutch-German borderland, *Int. J. Hyg. Environ. Health*, **208**, 373–381.
- Morgan, W.J. and Martinez, F.D. (1992) Risk factors for developing wheezing and asthma in childhood, *Pediatr. Clin. North Am.*, **39**, 1185–1203.
- Munir, A.K.M., Einarsson, R. and Schou, C. (1993) Allergens in school dust I. The amount of major cat (Fel d 1) and dog (Can f 1) allergens in dust from Swedish schools is high enough to probably cause perennial symptoms in most children with asthma who are sensitized to cat and dog, *J. Allergy Clin. Immunol.*, **91**, 1067–1074.
- von Mutius, E., Braun-Fahrlander, C., Schierl, R., Riedler, J., Ehlermann, S., Maisch, S., Waser, M. and Nowak, D. (2000) Exposure to endotoxin or other bacterial components might protect against the development of atopy, *Clin. Exp. Allergy*, **30**, 1230–1234.
- Nalli, S., Horn, O. J., Grochowalski, A.R., Copper, D.G. and Nicell, J. A. (2006) Origin of 2-ethylhexanol as a VOC, *Environ. Pollut.*, **140**, 181–185.
- Norbäck, D., Björnsson, E., Widström, J., Ström, G., Palmgren, U., Janson, C. and Boman, G. (1993) Asthma symptoms in relation to volatile organic compounds (VOC) and bacteria in dwellings. In: *Volatile Organic Compounds in the Environment '93: Proceeding of the International Conference on Volatile Organic Compounds in the Environment*, Oct 27–28, 1993, London, UK, 377–386.
- Norbäck, D., Björnsson, E., Janson, C., Widström, J. and Boman, G. (1995) Asthmatic symptoms and volatile organic compounds, formaldehyde, and carbon dioxide in dwellings, *Occup. Environ. Med.*, **52**, 388–395.
- Norbäck, D., Björnsson, E., Janson, C., Palmgren, U. and Boman, G. (1999) Current asthma and biochemical signs of inflammation in relation to building dampness in dwellings, *Int. J. Tuberc. Lung Dis.*, **3**, 368–376.
- Norbäck, D., Wieslander, G., Nordström, K. and Wålander, R. (2000) Asthma symptoms in relation to measured building dampness in upper concrete floor construction, and 2-ethyl-1-hexanol in indoor air, *Int. J. Tuberc. Lung Dis.*, **4**, 1016–1025.
- Norbäck, D., Mi, Y.H., Larson, L., Wady, L., Tao, J. and Mi, Y.L. (2002) Current asthma, respiratory infections and hypersensitivity to moulds in pupils in Shanghai, China, in relation to microbial components in the classrooms. In: *Indoor Air '02: Proceedings of the 9th International Conference on Indoor Air Quality and Climate*, June 30–July 5, 2002, Monterey, CA, USA, Vol. 3, 410–415.
- Oie, L., Hersoug, L.G. and Madsen, J.O. (1997) Residential exposure to plasticizers and its possible role in the pathogenesis of asthma, *Environ. Health Perspect.*, **105**, 972–978.
- Oie, L., Nafstad, P., Botten, G., Magnus, P. and Jaakkola, J.K. (1999) Ventilation in homes and bronchial obstruction in young children, *Epidemiology*, **10**, 294–299.
- Palmgren, U., Ström, G., Blomquist, G. and Malmberg, P. (1986) Collection of airborne micro-organisms on Nuclepore filters, estimation and analysis – CAMNEA method, *J. Appl. Bacteriol.*, **61**, 401–406.
- Pasanen, A.L., Korpi, A., Kasanen, J.P. and Pasanen, P. (1998) Critical aspects on the significance of microbial volatile metabolites as indoor air pollutants, *Environ. Int.*, **24**, 703–712.
- Plaschke, P.P., Janson, C., Norrman, E., Björnsson, E., Ellbjär, S. and Järholm, B. (2000) Onset and remission of allergic rhinitis and asthma and the relationship with atopic sensitization and smoking, *Am. J. Respir. Crit. Care Med.*, **162**, 920–924.
- Putus, T., Tuomainen, A. and Rautiala, S. (2004) Chemical and microbial exposures in a school building: adverse health effects in children, *Arch. Environ. Health*, **59**, 194–201.
- Riedler, J., Braun-Fahrlander, C., Eder, W., Schreuer, M., Waser, M., Maisch, S., Carr, D., Schierl, R., Nowak, D., von Mutius, E. and the ALEX Study Team. (2001) Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey, *Lancet*, **358**, 1129–1133.
- Rönmark, E., Lundbäck, B., Jönsson, E. and Platts-Mills, T. (1998) Asthma, type-1 allergy and related conditions in 7- and 8-year-old-children in Northern Sweden: prevalence rates and risk factor pattern, *Respir. Med.*, **92**, 316–324.
- Schleibinger, H., Laussmann, D., Brattig, C., Mangler, M., Eis, D. and Ruden, H. (2005) Emission patterns and emission rates of MVOC and the possibility for predicting hidden mold damage?, *Indoor Air*, **15** (Suppl. 9), 98–104.
- Sennhauser, F.H., Braun-Fahrlander, C. and Wildhaber, J.H. (2005) The burden of asthma in children: a European perspective, *Paediatr. Respir. Rev.*, **6**, 2–7.
- Shea, K.M. and the Committee on Environmental Health (2003) Pediatric exposure and potential toxicity of phthalate plasticizers, *Pediatrics*, **111**, 1467–1474.
- Simoni, M., Lombardi, E., Berti, G., Rusconi, F., La Grutta, S., Piffer, S., Petronio, M.G., Galassi, C., Forastiere, F., Viegi, G. and the SIDRIA-2 Collaborative Group. (2005) Mould/dampness exposure at home is associated with respiratory disorders in Italian children and adolescents: the SIDRIA-2 Study, *Occup. Environ. Med.*, **62**, 616–622.
- Skov, P., Valbjørn, O., Pedersen, B.V. and the Danish indoor climate study group (1990) Influence of indoor climate on the sick building syndrome in an office environment, *Scand. J. Work Environ. Health*, **16**, 363–371.
- Smedje, G. and Norbäck, D. (2001) Incidence of asthma diagnosis and self-reported allergy in relation to the school environment – a four-year follow-up study in schoolchildren, *Int. J. Tuberc. Lung Dis.*, **5**, 1059–1066.
- Smedje, G., Norbäck, D., Wessen, B. and Edling, C. (1996) Asthma among school employees in relation to the school environment. In: *Indoor Air '96: Proceedings of the 7th International Conference on Indoor Air Quality and Climate*, July 21–26, Nagoya, Japan, Vol. 1, 611–616.
- Smedje, G., Norbäck, D. and Edling, C. (1997) Asthma among secondary school pupils in relation to the school environment, *Clin. Exp. Allergy*, **27**, 1270–1278.
- Sparks, L.E., Guo, Z., Chang, J.C. and Tichenor, B.A. (1999) Volatile organic compound emissions from latex paint Part 2. Test house studies and indoor air quality (IAQ) modelling, *Indoor Air*, **9**, 18–25.
- Strachan, D.P. (1989) Hay fever, hygiene, and household size, *BMJ*, **299**, 1259–1260.
- Taskinen, T., Meklin, T., Nousiainen, M., Husman, T., Nevalainen, A. and Korppi, M. (1997) Moisture and mould problems in schools and respiratory manifestations in school children: clinical and skin test findings, *Acta Paediatr.*, **86**, 1181–1187.
- Tortolero, S.R., Bartholomew, L.K., Tyrell, S., Abramson, S.L., Sockrider, M.M., Markham, C.M., Whitehead, L.W. and Parcel, G.S. (2002) Environmental aller-

- gens and irritants in schools: a focus on asthma, *J. Sch. Health*, **72**, 33–38.
- Wålinder, R., Norbäck, D. and Johanson, G. (1998) Pulmonary reactions after exposure to 3-methylfuran vapour, a fungal metabolite, *Int. J. Tuberc. Lung Dis.*, **2**, 1037–1039.
- Wålinder, R., Ernstgård, L., Johanson, G., Norbäck, D., Venge, P. and Wieslander, G. (2005) Acute effects of a fungal volatile compound, *Environ. Health Perspect.*, **113**, 1775–1778.
- Wessen, B. and Schoeps, K.O. (1996) Microbial volatile organic compounds – what substances can be found in sick buildings? *Analyst*, **121**, 1203–1205.
- Wieslander, G., Norbäck, D., Björnsson, E., Janson, C. and Boman, G. (1997) Asthma and the indoor environment: the significance of emission of formaldehyde and volatile organic compounds from newly painted indoor surfaces, *Int. Arch. Occup. Environ. Health*, **69**, 115–124.
- Wieslander, G., Norbäck, D., Nordström, K., Wålinder, R. and Venge, P. (1999) Nasal and ocular symptoms, tear film stability and biomarkers in nasal lavage, in relation to building-dampness and building design in hospitals, *Int. Arch. Occup. Environ. Health*, **72**, 451–461.